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(54) Title: THE tagA GENE AND METHODS FOR DETECTING PREDISPOSITION TO PEPTIC ULCERATION

(57) Abstract

The present invention provides an isolated nucleic acid encoding an approximately 120-128 kilodalton antigen of *Helicobacter pylori*, or an antigenic fragment thereof, wherein the antigen is associated with peptic ulceration. The present invention also provides methods of detecting the presence of a *Helicobacter pylori* strain possessing the 120-128 kilodalton antigen in a subject, comprising the steps of contacting an antibody-containing sample from the subject with a detectable amount of the tagA antigen or antigenic fragment of the present invention and detecting the reaction of the antigen or fragment and the antibody. A mutant *H. pylori* not expressing a functional tagA antigen is also provided.

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" THE tagA GENE AND METHODS FOR DETECTING
PREDISPOSITION TO PEPTIC ULCERATION "

5

BACKGROUND OF THE INVENTION

10 Helicobacter pylori is now recognized as an important pathogen of humans in that the chronic gastritis it causes is a risk factor for the development of peptic ulcer disease and adenocarcinoma of the stomach. However, although essentially all infected persons develop gastritis, clinical consequences of H. pylori infection are recognized in only a minority of persons.

15 One explanation for this diversity of outcomes is that H. pylori strains may be heterogeneous. At a genetic level, H. pylori strains show a high degree of diversity, but most phenotypic characteristics are well-conserved. Two exceptions to the phenotypic homogeneity 20 are currently recognized. First, about 50%-60% of H. pylori strains produce a vacuolating cytotoxin in vitro (Cover et al. Infect. Immun. 58:603-610, 1990; Leunk et al. J. Med. Microbiol. 26:93-99, 1988), and toxin production is associated with peptic ulceration (Figura et 25 al. J. Clin. Microbiol. 27:225-226, 1988). Second, there is heterogeneity in whether an antigenic protein migrating at approximately 120-128 kilodalton (kDa) on reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis [SDS-PAGE] is produced (Cover et al. 58:603-610, 1990).
30 Although toxic activity is mediated by an 87 kDa protein (Cover and Blaser J. Biol. Chem. 267:10570-10575, 1992; Cover et al. J. Clin. Invest. 90: 913-918, 1992), toxin production itself is associated with the presence of the antigenic 120-128 kDa protein (Cover et al., 1990).
35 Previous studies have found that about 60-80% of H. pylori isolates express the 120-128 kDa protein (Apel et al. Zbl. Bakt. Hyg. A. 268:217-276, 1988; Cover et al., 1990). Notably, the presence of antibodies to the 120-128 kDa protein in either serum or mucosal secretions is

associated with the presence of peptic ulceration (Cover et al. 1990; Crabtree et al. Lancet 338:332-335).

Until now, little was known about the
5 association between toxin production and the 120-128 kDa antigen. This is due to the previous inability to further characterize the 120-128 kDa antigen after its initial visualization.

10 In previous studies, the 120-128 kDa antigen was visualized by Western blotting, but virtually no other characterization was performed (Cover et al., 1990). In contrast to the ease with which this antigen has been visualized by Western blotting, the 120-128 kDa band has
15 not been easily visualized by other methods such as silver staining (Figure 2 in Cover et al., 1990). The explanation for this phenomenon is that this antigen is present only in minute quantities, relative to other H. pylori proteins. Recently, Gerstenecker et al. (Eur. J. Clin. Microbiol. Infect. Dis. 11(7):595-601, 1992) have reported the isolation of an approximately 120 kDa protein from H. pylori which reacts with positive human control serum. However, virtually no characterization (such as N-terminal sequencing) of this antigen has been performed.

25

Despite the difficulty of purification, the present invention provides the cloning and sequence of the gene and deduced amino acid sequence of the 120-128 kDa protein. This data was obtained using alternate
30 methodology that did not require purification of the 120-128 kDa antigen. The invention also provides diagnostic, therapeutic, and prophylactic compositions and methods.

SUMMARY OF THE INVENTION

35

The present invention provides an isolated nucleic acid encoding an approximately 120-128 kilodalton

antigen of Helicobacter pylori, or an antigenic fragment thereof, wherein the antigen is associated with peptic ulceration. The present invention also provides methods of detecting the presence of a Helicobacter pylori strain 5 possessing the 120-128 kilodalton antigen in a subject, comprising the steps of contacting an antibody-containing sample from the subject with a detectable amount of the tagA antigen or fragment thereof of the present invention and detecting the reaction of the fragment and the 10 antibody.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows physical maps of plasmids pMC1, 15 pMC2, and pMC3. The large arrow beneath pMC3 represents the location of the tagA gene and the direction of transcription as determined by deletion mutations and immunoblotting. The small arrows represent the strand and extent of DNA sequenced from exonuclease III-derived 20 fragments. Restriction endonuclease cleavage sites: B, BglIII; Ba, BamHI; E, EcoRI; H, HindIII; N, NdeI; S, SacI.

Figure 2 shows a restriction map of pMC3:km used in construction of an H. pylori mutant. The km cassette 25 from pILL600 was ligated into the NdeI site of pMC3 to create pMC3:km. The arrows represent open reading frames including the truncated 2577 bp tagA open reading frame. Restriction sites are E, EcoRI; B, BglIII; H, HindIII; Ba, BamHI; N, NdeI; S, SacI; Sm, SmaI. pMC4 represents the 2.9 30 kb EcoRI to SacI fragment of pMC3.

Figure 3 shows physical maps of plasmids pMC3, pYB2 and pUT2. The large arrow beneath pUT2 represents the location of the tagA gene and the direction of 35 transcription as determined by deletion mutations and immunoblotting. Restriction endonuclease cleavage sites:

B, BalIII; Ba, BamHI; E, EcoRI; EV, EcoRV; H, HindIII; N, NdeI; S, SacI, X; XbaI.

DETAILED DESCRIPTION OF THE INVENTION

5

Nucleic Acids

The present invention provides an isolated nucleic acid encoding an approximately 120-128 kDa antigen or fragment of H. pylori, associated with peptic 10 ulceration. By "isolated" is meant separated from other nucleic acids found in the naturally occurring organism. The nucleic acid encoding the 120-128 kDa antigen is specific for H. pylori expressing the 120-128 kDa antigen. By "specific" is meant an isolated sequence which does not 15 hybridize with other nucleic acids to prevent an adequate positive hybridization with nucleic acids from H. pylori possessing the antigen. Specifically, an example of such a nucleic acid is an open reading frame of 3543 base pairs comprising nucleotides 1072 through 4614 contained in a 20 4821 base pair insert (SEQ ID NO:3). A cell line containing a plasmid having the full length tagA gene is deposited with the American Type Culture Collection (1230 Parklawn Drive, Rockville MD 20852) under ATCC Accession No. 69273. This specific nucleic acid can be used to 25 detect H. pylori possessing the 120-128 kDa antigen in methods such as polymerase chain reaction, ligase chain reaction and hybridization. Alternatively, the 4821 base pair sequence can be utilized to produce the full length tagA protein.

30

Another example of such a nucleic acid is a truncated open reading frame of 2577 base pairs comprising nucleotides 1072 through 3648 contained in a 3648 base pair insert (SEQ ID NO:1). This specific nucleic acid can 35 be used to detect H. pylori possessing the 120-128 kDa antigen in methods such as polymerase chain reaction, ligase chain reaction and hybridization. Alternatively,

5.

the 3648 base pair sequence can be utilized to produce a truncated protein.

In addition, the nucleic acid can be homologous
5 with nucleotide sequences present in other bacteria. Such
an amino acid sequence shared with other bacteria can be
used, for example, to simultaneously detect related
strains or as a basis for a multiprotective vaccine.

10 An isolated nucleic acid capable of selectively
hybridizing with or selectively amplifying a nucleic acid
encoding the 120-128 kDa antigen or fragments thereof is
also contemplated. An isolated nucleic acid complementary
to the above nucleic acid is also provided. The sequences
15 can be selected based on the nucleotide sequence and the
utility of the particular sequence.

Modifications to the nucleic acids of the
invention are also contemplated as long as the essential
20 structure and function of the polypeptide encoded by the
nucleic acids is maintained. Likewise, fragments used as
primers or probes can have substitutions so long as enough
complementary bases exist for selective hybridization
(Kunkel et al. Methods Enzymol. 1987:154:367, 1987).

25

Antigen

Purified antigenic polypeptide fragments encoded
by the nucleic acids of the present invention are also
contemplated. As used herein, "purified" means the
30 antigen is sufficiently free of contaminants or cell
components with which the antigen normally occurs to
distinguish the antigen from the contaminants or
components. The purified approximately 120-128 kDa full-
length antigen, truncated antigen and antigenic fragments
35 of the present invention are also referred to herein as
"the antigen" or "the tagA antigen."

Specifically provided is an approximately 130 kDa full length tagA antigenic polypeptide (SEQ ID NO:4), encoded by an open reading frame of 3543 base pairs within the 4821 base pair cloned insert, consisting essentially of the amino acids encoded by nucleotides 1072 through 4614 contained in the nucleotide sequence defined in the Sequence Listing as SEQ ID NO:3.

Additionally, an approximately 96 kDa antigenic polypeptide is encoded by an open reading frame of 2577 base pairs within the 3648 base pair cloned insert, consisting essentially of the amino acids encoded by nucleotides 1072 through 3648 contained in the nucleotide sequence defined in the Sequence Listing as SEQ ID NO:1.

15

An antigenic fragment of the antigen can be isolated from the whole antigen or truncated antigen by chemical or mechanical disruption. The purified fragments thus obtained can be tested to determine their antigenicity and specificity by the methods taught herein. Antigenic fragments of the antigen can also be synthesized directly. An immunoreactive fragment is defined as an amino acid sequence of at least about 5 consecutive amino acids derived from the antigen amino acid sequence.

25

The polypeptide fragments of the present invention can also be recombinant proteins obtained by cloning nucleic acids encoding the polypeptide in an expression system capable of producing the antigenic polypeptide or fragments thereof.

Once the amino acid sequence of the antigen is provided, it is also possible to synthesize, using standard peptide synthesis techniques, peptide fragments chosen to be homologous to immunoreactive regions of the antigen and to modify these fragments by inclusion, deletion or modification of particular amino acids

residues in the derived sequences. Thus, synthesis or purification of an extremely large number of peptides derived from the antigen is possible.

5 The amino acid sequences of the present polypeptides can contain an immunoreactive portion of tagA antigen attached to sequences designed to provide some additional property, such as solubility. The amino acid sequences of a tagA antigen can include sequences in which
10 one or more amino acids have been substituted with another amino acid to provide some additional property, such as to remove/add amino acids capable of disulfide bonding, to increase its bio-longevity, alter enzymatic activity, or alter interactions with gastric acidity. In any case, the
15 peptide must posses a bioactive property, such as immunoreactivity, immunogenicity, etc.

Determining Immunogenicity

The purified polypeptide fragments thus obtained
20 can be tested to determine their immunogenicity and specificity. Briefly, various concentrations of a putative immunogenically specific fragment are prepared and administered to an animal and the immunological response (e.g., the production of antibodies or cell
25 mediated immunity) of the animal to each concentration is determined. The amounts of antigen administered depend on the subject, e.g. a human or a guinea pig, the condition of the subject, the size of the subject, etc. Thereafter an animal so inoculated with the antigen can be exposed to
30 the bacterium to test the potential vaccine effect of the specific immunogenic fragment. The specificity of a putative immunogenic fragment can be ascertained by testing sera, other fluids or lymphocytes from the inoculated animal for cross reactivity with other closely
35 related bacteria.

Vectors and Hosts

A vector comprising the nucleic acids of the present invention is also provided. The vectors of the invention can be in a host capable of expressing the 5 antigen.

There are numerous E. coli expression vectors known to one of ordinary skill in the art useful for the expression of the antigen. Other microbial hosts suitable 10 for use include bacilli, such as Bacillus subtilis, and other Enterobacteriaceae, such as Salmonella, Serratia, and various Pseudomonas species. In these prokaryotic hosts one can also make expression vectors, which will typically contain expression control sequences compatible 15 with the host cell (e.g., an origin of replication). In addition, any number of a variety of well-known promoters will be present, such as the lactose promoter system, a tryptophan (Trp) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. 20 The promoters will typically control expression, optionally with an operator sequence, and have ribosome binding site sequences for example, for initiating and completing transcription and translation. If necessary an amino terminal methionine can be provided by insertion of 25 a Met codon 5' and in-frame with the antigen. Also, the carboxy-terminal extension of the antigen can be removed using standard oligonucleotide mutagenesis procedures.

Additionally, yeast expression can be used.

30 There are several advantages to yeast expression systems. First, evidence exists that proteins produced in a yeast secretion systems exhibit correct disulfide pairing. Second, post-translational glycosylation is efficiently carried out by yeast secretory systems. The Saccharomyces 35 cerevisiae pre-pro-alpha-factor leader region (encoded by the MFa-1 gene) is routinely used to direct protein secretion from yeast (Brake et al., 1984). The leader

region of pre-pro-alpha-factor contains a signal peptide and a pro-segment which includes a recognition sequence for a yeast protease encoded by the KEX2 gene: this enzyme cleaves the precursor protein on the carboxyl side 5 of a Lys-Arg dipeptide cleavage-signal sequence. The antigen coding sequence can be fused in-frame to the pre-pro-alpha-factor leader region. This construct is then put under the control of a strong transcription promoter, such as the alcohol dehydrogenase I promoter or a 10 glycolytic promoter. The antigen coding sequence is followed by a translation termination codon which is followed by transcription termination signals. Alternatively, the antigen coding sequences can be fused to a second protein coding sequence, such as Sj26 or β - 15 galactosidase, used to facilitate purification of the fusion protein by affinity chromatography. The insertion of protease cleavage sites to separate the components of the fusion protein is applicable to constructs used for expression in yeast.

20

Mammalian cells permit the expression of proteins in an environment that favors important post-translational modifications such as folding and cysteine pairing, addition of complex carbohydrate structures, and 25 secretion of active protein. Vectors useful for the expression of antigen in mammalian cells are characterized by insertion of the antigen coding sequence between a strong viral promoter and a polyadenylation signal. The vectors can contain genes conferring either gentamicin or 30 methotrexate resistance for use as selectable markers. The antigen and immunoreactive fragment coding sequence can be introduced into a Chinese hamster ovary cell line using a methotrexate resistance-encoding vector. Presence of the vector DNA in transformed cells can be confirmed by 35 Southern analysis and production of an RNA corresponding to the antigen coding sequence can be confirmed by Northern analysis. A number of other suitable host cell

lines capable of secreting intact human proteins have been developed in the art, and include the CHO cell lines, HeLa cells, myeloma cell lines, Jurkat cells, etc. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, an enhancer, and necessary information processing sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Preferred expression control sequences are promoters derived from immunoglobulin genes, SV40, Adenovirus, Bovine Papilloma Virus, etc. The vectors containing the DNA segments of interest can be transferred into the host cell by well-known methods, which vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment or electroporation may be used for other cellular hosts.

Alternative vectors for the expression of antigen in mammalian cells, those similar to those developed for the expression of human gamma-interferon, tissue plasminogen activator, clotting Factor VIII, hepatitis B virus surface antigen, protease NexinI, and eosinophil major basic protein, can be employed. Further, the vector can include CMV promoter sequences and a polyadenylation signal available for expression of inserted DNAs in mammalian cells (such as COS7).

The DNA sequences can be expressed in hosts after the sequences have been operably linked to, i.e., positioned to ensure the functioning of, an expression control sequence. These expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors can contain selection markers, e.g., tetracycline resistance or hygromycin resistance, to permit detection and/or selection of those cells

transformed with the desired DNA sequences (see, e.g., U.S. Patent 4,704,362).

Polynucleotides encoding a variant polypeptide

5 may include sequences that facilitate transcription (expression sequences) and translation of the coding sequences such that the encoded polypeptide product is produced. Construction of such polynucleotides is well known in the art. For example, such polynucleotides can
10 include a promoter, a transcription termination site (polyadenylation site in eukaryotic expression hosts), a ribosome binding site, and, optionally, an enhancer for use in eukaryotic expression hosts, and, optionally, sequences necessary for replication of a vector.

15

Purified Antibodies

A purified monoclonal antibody specifically reactive with the antigen is also provided. The antibodies can be specifically reactive with a unique
20 epitope of the antigen or they can also react with epitopes of other organisms. The term "reactive" means capable of binding or otherwise associating nonrandomly with an antigen. "Specifically reactive" as used herein describes an antibody or other ligand that does not cross
25 react substantially with any antigen other than the one specified, in this case, the tagA antigen. Antibodies can be made as described in the Examples (see also, Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1988). Briefly
30 purified antigen can be injected into an animal in an amount and in intervals sufficient to elicit an immune response. Antibodies can either be purified directly, or spleen cells can be obtained from the animal. The cells are then fused with an immortal cell line and screened for
35 antibody secretion.

The antibody can be bound to a substrate or labeled with a detectable moiety or both bound and labeled. The detectable moieties contemplated with the composition of the present invention are those listed 5 below in the description of the diagnostic methods, including fluorescent, enzymatic and radioactive markers.

Antigen Bound to Substrate

A purified tagA antigen bound to a substrate and 10 a ligand specifically reactive with the antigen are also contemplated. Such a purified ligand specifically reactive with the antigen can be an antibody. The antibody can be a monoclonal antibody obtained by standard methods and as described herein. The monoclonal antibody 15 can be secreted by a hybridoma cell line specifically produced for that purpose (Harlow and Lane, 1988). Likewise, nonhuman polyclonal antibodies specifically reactive with the antigen are within the scope of the present invention. The polyclonal antibody can also be 20 obtained by the standard immunization and purification protocols (Harlow and Lane, 1988).

Serological Detection (Diagnosis) Methods

Detecting Antibody with Antigen

25 The present invention provides a method of detecting the presence of a H. pylori strain possessing the 120-128 kDa antigen in a subject, comprising the steps of contacting an antibody-containing sample from the subject with a detectable amount of the tagA or tagA 30 antigenic fragment of the present invention and detecting the reaction of the tagA or fragment and the antibody, the reaction indicating the presence of the toxic H. pylori strain or previous infection with the toxic H. pylori strain.

Detecting Antigen with Antibody/Ligand

One example of the method of detecting H. pylori possessing the antigen is performed by contacting a fluid or tissue sample from the subject with an amount of a 5 purified antibody specifically reactive with the antigen, and detecting the reaction of the ligand with the antigen. It is contemplated that the antigen will be on intact cells containing the antigen, or will be fragments of the antigen. As contemplated herein, the antibody includes 10 any ligand which binds the antigen, for example, an intact antibody, a fragment of an antibody or another reagent that has reactivity with the antigen. The fluid sample of this method can comprise any body fluid which would contain the antigen or a cell containing the antigen, such 15 as blood, plasma, serum, saliva and urine. Other possible examples of body fluids include sputum, mucus, gastric juice and the like.

ELISA

20 Immunoassays such as immunofluorescence assays (IFA), enzyme linked immunosorbent assays (ELISA) and immunoblotting can be readily adapted to accomplish the detection of the antigen. An ELISA method effective for the detection of the antigen can, for example, be as 25 follows: (1) bind the antibody to a substrate; (2) contact the bound antibody with a fluid or tissue sample containing the antigen; (3) contact the above with a secondary antibody bound to a detectable moiety (e.g., horseradish peroxidase enzyme or alkaline phosphatase 30 enzyme); (4) contact the above with the substrate for the enzyme; (5) contact the above with a color reagent; (6) observe color change. The above method can be readily modified to detect antibody as well as antigen. A specific example of an ELISA of the present invention is 35 provided in Example 5.

Competitive Inhibition Assay

Another immunologic technique that can be useful in the detection of H. pylori expressing tagA or previous H. pylori infection utilizes monoclonal antibodies (MAbs) 5 for detection of antibodies specifically reactive with tagA antigen. Briefly, sera or other body fluids from the subject is reacted with the antigen bound to a substrate (e.g. an ELISA 96-well plate). Excess sera is thoroughly washed away. A labeled (enzyme-linked, fluorescent, 10 radioactive, etc.) monoclonal antibody is then reacted with the previously reacted antigen-serum antibody complex. The amount of inhibition of monoclonal antibody binding is measured relative to a control (no patient serum antibody). The degree of monoclonal antibody 15 inhibition is a very specific test for a particular variety or strain since it is based on monoclonal antibody binding specificity. MAbs can also be used for detection directly in cells by IFA.

20 Micro-Agglutination Assay

A micro-agglutination test can also be used to detect the presence of the tagA-possessing H. pylori strain in a subject. Briefly, latex beads (or red blood cells) are coated with the antigen and mixed with a sample 25 from the subject, such that antibodies in the tissue or body fluids that are specifically reactive with the antigen crosslink with the antigen, causing agglutination. The agglutinated antigen-antibody complexes form a precipitate, visible with the naked eye or by 30 spectrophotometer. In a modification of the above test, antibodies specifically reactive with the antigen can be bound to the beads and antigen in the tissue or body fluid thereby detected.

35 Sandwich Assay/Flow Cytometry/Immunoprecipitation

In addition, as in a typical sandwich assay, the antibody can be bound to a substrate and reacted with the

antigen. Thereafter, a secondary labeled antibody is bound to epitopes not recognized by the first antibody and the secondary antibody is detected. Since the present invention provides tagA antigen for the detection of toxic 5 H. pylori or previous H. pylori infection other serological methods such as flow cytometry and immunoprecipitation can also be used as detection methods.

In the diagnostic methods taught herein, the 10 antigen can be bound to a substrate and contacted by a fluid sample such as serum, urine, saliva or gastric juice. This sample can be taken directly from the patient or in a partially purified form. In this manner, antibodies specific for the antigen (the primary antibody) 15 will specifically react with the bound antigen.

Thereafter, a secondary antibody bound to, or labeled with, a detectable moiety can be added to enhance the detection of the primary antibody. Generally, the secondary antibody or other ligand which is reactive, 20 either specifically with a different epitope of the antigen or nonspecifically with the ligand or reacted antibody, will be selected for its ability to react with multiple sites on the primary antibody. Thus, for example, several molecules of the secondary antibody can 25 react with each primary antibody, making the primary antibody more detectable.

Detectable Moieties

The detectable moiety will allow visual 30 detection of a precipitate or a color change, visual detection by microscopy, or automated detection by spectrometry, radiometric measurement or the like. Examples of detectable moieties include fluorescein and rhodamine (for fluorescence microscopy), horseradish 35 peroxidase (for either light or electron microscopy and biochemical detection), biotin-streptavidin (for light or electron microscopy) and alkaline phosphatase (for

biochemical detection by color change). The detection methods and moieties used can be selected, for example, from the list above or other suitable examples by the standard criteria applied to such selections (Harlow and 5 Lane, 1988).

Detecting Disease

Because the purified tagA antigen provided herein is associated with peptic ulceration, the present 10 invention also provides a method of detecting predisposition to peptic ulceration in a subject. The method can be accomplished according to the methods set forth above for the detection of H. pylori expressing the tagA antigen or for the detection of antibodies specific 15 to the tagA antigen or for the detection of specific antibodies to the tagA antigen. The presence of the tagA antigen or tagA specific antibodies indicates a predisposition of the subject to peptic ulceration.

20 Treatment Methods

Methods of treating peptic ulcers in a subject using the compositions of the present invention are provided. For example, in one such method an amount of ligand specifically reactive with the approximately 120- 25 128 kDa antigen of H. pylori sufficient to bind the antigen in the subject and improve the subject's clinical condition is administered to the subject. Such improvement results from the ligand interfering with the antigen's normal function in inducing inflammation and 30 cellular damage. The ligand can be a purified monoclonal antibody specifically reactive with the antigen, a purified polyclonal antibody derived from a nonhuman animal, or other reagent having specific reactivity with the antigen. Additionally, cytotoxic moieties can be 35 conjugated to the ligand/antibody by standard methods. Examples of cytotoxic moieties include ricin A chain, diphtheria toxin and radioactive isotopes.

Another method of treating peptic ulcers in a subject comprises administering to the subject an amount of a ligand/antagonist for a receptor for the 120-128 kDa antigen of H. pylori sufficient to react with the receptor and prevent the binding of the 120-128 kDa antigen to the receptor. The result is an improvement in the subject's clinical condition. Alternatively, the treatment method can include administering to the subject an amount of an analogue of a tagA receptor to result in competitive binding of the tagA antigen, thus inhibiting binding of the tagA antigen to its wild type receptor. The receptor is localized on cells present in the gastroduodenal mucosa, such as epithelial cells, inflammatory cells, or endothelial cells.

15

Mutant Organism

The present invention also provides a mutant H. pylori in which the tagA gene product has been rendered nonfunctional. In one example, the mutant H. pylori strain is obtained by making a substitution mutation in the coding sequence for the tagA antigen as described in the Examples. Since the present invention provides the nucleic acid encoding the antigen, other methods of mutating the coding sequence of the antigen can be used to obtain other mutant strains as contemplated herein. An example of the mutant H. pylori strain of the present invention is designated 84-183:M22 and is deposited with the American Type Culture Collection (1230 Parklawn Drive, Rockville, MD 20852) under ATCC Accession Number 55359.

30

Additional isogenic mutants can be prepared, for example, by inserting a nucleic acid in the tagA gene or deleting a portion of the tagA gene so as to render the gene non-functional or produced in such low amounts that the organism is non-infectious. Furthermore, by providing the nucleotide sequence for the nucleic acid encoding the antigen, the present invention permits the making of

specific point mutations having the desired effect. The deletion, insertion or substitution mutations can be made in the gene sequence in either the regulatory or coding region to prevent transcription or to render the
5 transcribed product nonfunctional.

One such approach to the construction of a deletion or insertion mutant is via the Donnenberg method (Donnenberg and Kaper Infect. Immun. 4310-4317, 1991). A
10 deletion in tagA is created by deleting a 0.2 kb BamH1-NdeI fragment and religating the tagA clone. This mutant is cloned into suicide vector pILL570. The sacB gene of Bacillus subtilis is also cloned into the suicide vector to provide a conditionally lethal phenotype. This
15 construct is transformed into H. pylori by electroporation, and transformants selected by spectinomycin resistance. The merodiploid strain which contains the suicide vector and the mutated version of the tagA gene are exposed to sucrose to directly select for
20 organisms that have undergone a second recombination, resulting in the loss of the vector. These and other well known methods of making mutations can be applied to the nucleic acids provided herein to obtain other desired mutations.

25

Vaccines

The antigen or mutant H. pylori of this invention can be used in the construction of a vaccine comprising an immunogenic amount of the antigen or mutant
30 H. pylori and a pharmaceutically acceptable carrier. The vaccine can be the entire antigen, the antigen on an intact H. pylori, E. coli or other strain. The vaccine can then be used in a method of preventing peptic ulceration or other complications of H. pylori infection
35 (including atrophic gastritis and malignant neoplasms of the stomach).

Immunogenic amounts of the antigen can be determined using standard procedures. Briefly, various concentrations of a putative specific immunoreactive epitope are prepared, administered to an animal and the 5 immunological response (e.g., the production of antibodies) of an animal to each concentration is determined.

The pharmaceutically acceptable carrier in the 10 vaccine of the instant invention can comprise saline or other suitable carriers (Arnon, R. (Ed.) Synthetic Vaccines I:83-92, CRC Press, Inc., Boca Raton, Florida, 1987). An adjuvant can also be a part of the carrier of the vaccine, in which case it can be selected by standard 15 criteria based on the antigen used, the mode of administration and the subject (Arnon, R. (Ed.), 1987). Methods of administration can be by oral or sublingual means, or by injection, depending on the particular vaccine used and the subject to whom it is administered.

20

It can be appreciated from the above that the vaccine can be used as a prophylactic or a therapeutic modality. Thus, the invention provides methods of preventing or treating H. pylori infection and the 25 associated diseases by administering the vaccine to a subject.

Nucleic Acid Detection (Diagnosis) Methods

The presence of the tagA antigen and H. pylori 30 possessing the tagA antigen can also be determined by detecting the presence of a nucleic acid specific for the antigen. The specificity of these sequences for the antigen can be determined by conducting a computerized comparison with known sequences, catalogued in GenBank, a 35 computerized database, using the computer programs Word Search or FASTA of the Genetics Computer Group (Madison,

WI), which search the catalogued nucleotide sequences for similarities to the gene in question.

The nucleic acid specific for the antigen can be

5 detected utilizing a nucleic acid amplification technique, such as polymerase chain reaction or ligase chain reaction. Alternatively, the nucleic acid is detected utilizing direct hybridization (e.g., the colony blot method shown Example 4) or by utilizing a restriction

10 fragment length polymorphism. For example, the present invention provides a method of detecting the presence of H. pylori, possessing the tagA antigen, comprising ascertaining the presence of a nucleotide sequence associated with a restriction endonuclease cleavage site.

15 In addition, PCR primers which hybridize only with nucleic acids specific for the antigen can be utilized. The presence of amplification indicates the presence of the antigen. In another embodiment a restriction fragment of a DNA sample can be sequenced directly using, for example,

20 Sanger ddNTP sequencing or 7-deaza-2'-deoxyguanosine 5'-triphosphate and Taq polymerase and compared to the known unique sequence to detect H. pylori. In a further embodiment, the present invention provides a method of detecting the presence of tagA- containing H. pylori by

25 selective amplification by the methods described above. In yet another embodiment H. pylori can be detected by directly hybridizing the unique sequence with a tagA selective nucleic acid probe. Furthermore, the nucleotide sequence could be amplified prior to hybridization by the

30 methods described above.

Once specific variable sequences are shown to be associated with peptic ulceration, the methods to detect these sequences are standard in the art. Detection of

35 point mutations or variable sequences using direct probing involves the use of oligonucleotide probes which may be prepared, for example, synthetically or by nick

translation. The probes may be suitably labeled using, for example, a radio label, enzyme label, fluorescent label, biotin-avidin label and the like for subsequent visualization in the example of Southern blot

5 hybridization procedure. The labeled probe is reacted with a bound sample DNA, e.g., to a nitrocellulose sheet under conditions such that only fully complementary sequences hybridize. The areas that carry DNA sequences complementary to the labeled DNA probe become labeled
10 themselves as a consequence of the reannealing reaction. The areas of the filter that exhibit such labeling may then be visualized, for example, by autoradiography. The labeled probe is reacted with a DNA sample bound to, for example, nitrocellulose under conditions such that only
15 fully complementary sequences will hybridize. The stringency of hybridization is usually 5°C below the Ti (the irreversible melting temperature of the hybrid formed between the probe and its target sequence) for the given chain length. For 20mers the recommended hybridization
20 temperature is about 58°C. The washing temperatures are unique to the sequence under investigation and need to be optimized for each variant.

Alternative probing techniques, such as ligase
25 chain reaction (LCR), involve the use of mismatch probes, i.e., probes which are fully complementary with the target except at the point of the mutation. The target sequence is then allowed to hybridize both with oligonucleotides which are fully complementary and have oligonucleotides
30 containing a mismatch, under conditions which will distinguish between the two. By manipulating the reaction conditions, it is possible to obtain hybridization only where there is full complementarity. If a mismatch is present there is significantly reduced hybridization.

35

The polymerase chain reaction (PCR) is a technique that amplifies specific DNA sequences with

remarkable efficiency. Repeated cycles of denaturation, primer annealing and extension carried out with polymerase, e.g., a heat stable enzyme Tag polymerase, leads to exponential increases in the concentration of

5 desired DNA sequences. Given a knowledge of the nucleotide sequence of a mutation, synthetic oligonucleotides can be prepared which are complementary to sequences which flank the DNA of interest. Each oligonucleotide is complementary to one of the two

10 strands. The DNA can be denatured at high temperatures (e.g., 95°C) and then reannealed in the presence of a large molar excess of oligonucleotides. The oligonucleotides, oriented with their 3' ends pointing towards each other, hybridize to opposite strands of the

15 target sequence and prime enzymatic extension along the nucleic acid template in the presence of the four deoxyribonucleotide triphosphates. The end product is then denatured again for another cycle. After this three-step cycle has been repeated several times, amplification

20 of a DNA segment by more than one million-fold can be achieved. The resulting DNA may then be directly sequenced in order to locate any genetic alteration.

Alternatively, it may be possible to prepare oligonucleotides that will only bind to altered DNA, so

25 that PCR will only result in multiplication of the DNA if a mutation is present. Following PCR, direct visualization or allele-specific oligonucleotide hybridization may be used to detect disease associated with a point mutation. Alternatively, an adaptation of

30 PCR called amplification of specific alleles (PASA) can be employed; this uses differential amplification for rapid and reliable distinction between alleles that differ at a single base pair. Other techniques, such as 3SR, which utilize RNA polymerase to achieve high copy number, can

35 also be used where appropriate.

In yet another method, PCR may be followed by restriction endonuclease digestion with subsequent analysis of the resultant products. Nucleotide substitutions can result in the gain or loss of specific 5 restriction endonuclease site. The gain or loss of a restriction endonuclease recognition site facilitates the detection of the disease associated mutation using restriction fragment length polymorphism (RFLP) analysis or by detection of the presence or absence of a 10 polymorphic restriction endonuclease site in a PCR product that spans the sequence of interest.

For RFLP analysis, DNA is obtained, for example from the blood, gastric specimen, saliva, dental plaque, 15 other bodily fluids or stool of the subject suspected of containing tagA-possessing H. pylori, or H. pylori isolated from subject, and from a subject infected with nontoxic H. pylori, is digested with a restriction endonuclease, and subsequently separated on the basis of 20 size by agarose gel electrophoresis. The Southern blot technique can then be used to detect, by hybridization with labeled probes, the products of endonuclease digestion. The patterns obtained from the Southern blot can then be compared. Using such an approach, tagA DNA is 25 detected by determining the number of bands detected and comparing this number to the DNA from H. pylori strains that are not associated with severe disease. Restriction endonucleases can also be utilized effectively to detect mutations in the tagA gene.

30

Similar creation of additional restriction sites by nucleotide substitutions at the disclosed mutation sites can be readily calculated by reference to the genetic code and a list of nucleotide sequences recognized 35 by restriction endonucleases.

Single strand conformational analysis (SSCA) offers a relatively quick method of detecting sequence changes which may be appropriate in at least some instances.

5

In general, primers for PCR and LCR are usually about 20 bp in length and the preferable range is from 15-25 bp. Better amplification is obtained when both primers are the same length and with roughly the same nucleotide 10 composition. Denaturation of strands usually takes place at 94°C and extension from the primers is usually at 72°C. The annealing temperature varies according to the sequence under investigation. Examples of reaction times are: 20 mins denaturing; 35 cycles of 2 min, 1 min, 1 min for 15 annealing, extension and denaturation; and finally a 5 min extension step.

PCR amplification of specific alleles (PASA) is a rapid method of detecting single-base mutations or 20 polymorphisms. PASA (also known as allele specific amplification) involves amplification with two oligonucleotide primers such that one is allele-specific. The desired allele is efficiently amplified, while the other allele(s) is poorly amplified because it mismatches 25 with a base at or near the 3' end of the allele-specific primer. Thus, PASA or the related method of PAMSA may be used to specifically amplify the mutation sequences of the invention. Where such amplification is done on H. pylori isolates or samples obtained from an individual, it can 30 serve as a method of detecting the presence of the mutations.

As mentioned above, a method known as ligase chain reaction (LCR) can be used to successfully detect a 35 single-base substitution. LCR probes may be combined or multiplexed for simultaneously screening for multiple different mutations. Thus, LCR can be particularly useful

where, as here, multiple mutations are predictive of the same disease.

Antigen-Detecting Kit

5 The present invention provides a kit for the diagnosis of infection by strains of H. pylori possessing the tagA antigen. Particularly, the kit can detect the presence of tagA antigen specifically reactive with an antibody or an immunoreactive fragment thereof. The kit
10 can include an antibody bound to a substrate, a secondary antibody reactive with the antigen and a reagent for detecting a reaction of the secondary antibody with the antigen. Such a kit can be an ELISA kit and can comprise the substrate, primary and secondary antibodies when
15 appropriate, and any other necessary reagents such as detectable moieties, enzyme substrates and color reagents as described above. The diagnostic kit can, alternatively, be an immunoblot kit generally comprising the components and reagents described herein.

20

Antibody-Detecting Kit

The diagnostic kit of the present invention can be used to detect the presence of a primary antibody specifically reactive with tagA or an antigenic fragment thereof. The kit can include the antigen bound to a substrate, a secondary antibody reactive with the antibody specifically reactive with the tagA antigen and a reagent for detecting a reaction of the secondary antibody with the primary antibody. Such a kit can be an ELISA kit and
25 can comprise the substrate, antigen, primary and secondary antibodies when appropriate, and any other necessary reagents such as detectable moieties, enzyme substrates and color reagents as described above. The diagnostic kit can, alternatively, be an immunoblot kit generally
30 comprising the components and reagents described herein.
35

Nucleic Acid Detection (Diagnostic) Kits

Once the nucleotide sequence of the tagA antigen is determined, the diagnostic kit of the present invention can alternatively be constructed to detect nucleotide sequences specific for the antigen comprising the standard kit components such as the substrate and reagents for the detection of nucleic acids. Because H. pylori infection can be diagnosed by detecting nucleic acids specific for the antigen in gastric or duodenal tissue and body fluids such as gastric juice, urine, stool, and saliva, it will be apparent to an artisan that a kit can be constructed that utilizes the nucleic acid detection methods, such as specific nucleic acid probes, primers or restriction fragment length polymorphisms in analyses. It is contemplated that the diagnostic kits will further comprise a positive and negative control test.

The particular reagents and other components included in the diagnostic kits of the present invention can be selected from those available in the art in accord with the specific diagnostic method practiced in the kit. Such kits can be used to detect the antigen in tissue and fluid samples from a subject.

The following examples are intended to illustrate, but not limit, the invention. While they are typical of those that might be used, other procedures known to those skilled in the art may be alternatively employed.

30

EXAMPLE 1**Cloning and Expression of tagA Antigen**

Bacterial strains and growth conditions.

H. pylori strain 84-183 (ATCC 53726) was used to clone the gene for the tagA antigen. Thirty-two clinical

H. pylori isolates from humans, including strains that had been previously shown to possess the antigen, were used to assess conservation of the gene and correlation with cytotoxin production (Table 1). Stock cultures were 5 maintained at -70°C in Brucella broth (BBL Microbiology Systems, Cockeysville, MD) supplemented with 15% glycerol. H. pylori strains were cultured in Brucella broth supplemented with 5% fetal bovine serum in a microaerobic atmosphere (generated by CampyPak-Plus (BBL) at 37°C for 10 48 hours. For transformation and protein expression, E. coli strains XL1-Blue (Stratagene, La Jolla, CA), HB101 (ATCC 33694), and DH5 α (Stratagene, La Jolla, CA) were cultured in Luria-Bertoli (LB) medium with shaking at 37°C. The final concentrations of ampicillin when added 15 to media was 100 μ g/ml.

Chemicals and enzymes.

Isopropyl- β -D-thiogalactopyranoside (IPTG) was purchased from Sigma Chemical Co. (St. Louis, MO) and used 20 at 57 μ g/ml, and 5-bromo-4-chloro-3-indolyl- β -D-galactoside (X-GAL; final concentration 40 μ g/ml) was from Boehringer-Mannheim (Indianapolis, IN). Restriction enzymes, T4 DNA ligase, E. coli DNA polymerase large (Klenow) fragment and Sequenase™ were from Promega and 25 United States Biochemicals (Cleveland, OH). [α - 32 P] dATP (650 Ci/mmol) was from ICN Radiochemicals (Irvine, CA).

Genetic techniques and nucleotide sequence analysis.

To obtain chromosomal DNA from H. pylori 84-183, 30 the strain was cultured for 48 h in Brucella broth containing 5% fetal bovine serum, the cells pelleted, and resuspended in 100 mM Tris-HCl (pH 7.2) containing 100mM NaCl. Cells were lysed using 1% SDS in 100 mM Tris-HCl (pH 8.8). After chloroform-phenol extractions, the 35 chromosomal DNA was precipitated with 100% ethanol. Plasmids were isolated by the rapid alkaline extraction procedure of Birnboim and Doly (Nucleic Acids. Res.,

7:1513-1523, 1979) and purification was completed by precipitation in the presence of 800 mM NaCl and 6.5% polyethylene glycol. All other standard molecular genetic techniques, including sequential ordered deletions, were

5 performed as described (Sambrook et al. Molecular cloning: A Laboratory Manual, 1989). The nucleotide sequence was determined unambiguously on both strands using double-stranded DNA templates and the dideoxy chain termination procedure as described previously (Sanger et al. Proc.

10 Natl. Acad. Sci. U.S.A., 71:1342-1346.32, 1977).

Oligonucleotide primers were synthesized by the Vanderbilt University DNA Core Facility using a Milligen 7500 DNA synthesizer, using the manufacturer's protocol.

15 Nucleotide sequences were compiled and analyzed with the aid of the DNA-Star program (DNA Star, Inc., Madison, WI); putative promoter and Shine-Dalgarno sequences were identified by comparison with consensus sequences (Hawley and McClure Nucleic Acids Res., 11:2237-2255, 1983).

20 **Construction of a genomic library from H. pylori.**

Strain 84-183 chromosomal DNA was sheared by sonication and the resulting fragments were electrophoresed on a 0.7% low melting temperature agarose gel. Fragments in the 2-10 kb size range were excised,

25 treated with T4 DNA polymerase to produce blunt ends, and ligated to phosphorylated EcoRI octamer linkers (New England Biolabs, Beverly, MA). The DNA was digested with EcoRI and ligated to the EcoRI arms of the λ ZapII vector, according to the manufacturer's protocol. The ligation

30 mixtures were added to the Gigapack IIa packaging mix (Stratagene) and titered on XL1-blue cells (λ ZapII) or Y1088 (λ gt11) cells. The amplified phage libraries were screened with adsorbed sera from an H. pylori-infected person or by plaque hybridization.

Cloning of *H. pylori*-specific genes.

Serum from an *H. pylori*-infected person that strongly recognizes the 120-128 kDa antigen was adsorbed with *H. pylori* strain 86-313, which does not produce the 5 120-128 kDa band, and with *E. coli* cells to reduce the likelihood of nonspecific reactivity and then used to screen a bank of genes from the amplified λ ZapII phage library (Blaser and Gotschlich *J. Biol. Chem.* 265:14529-14535, 1990). The bank contained approximately 4×10^4 10 insertions. The amplified phage library was screened by allowing approximately 10^5 plaques to grow on XL1 Blue cells for 2.5 h at 42°C, overlaying with a nitrocellulose filter previously impregnated with 10 mM IPTG, and incubating for 2 h at 37°C. The filters were then 15 screened with the adsorbed serum to detect 9 reactive clones. Positive plaques were then plaque purified, and lysates were prepared from these infected *E. coli* cells. The lysates were immunoblotted with the adsorbed serum and clones expressing recombinant proteins were saved. By 20 immunoblotting with the adsorbed human serum, each of the XL1-Blue lysates showed a strongly immunoreactive band migrating at either approximately 75, 85, or 96 kDa, corresponding to plasmids pMC1, pMC2, or pMC3, respectively.

25

From the three representative clones, the pBluescript plasmids containing the cloned DNA inserts were excised by co-infection with helper phage, as detailed (Short et al. *Nucleic Acids Res.*, 16:7583-7600, 1988), and fresh XL1-30 Blue cells transformed. After plasmid purification, restriction enzyme cleavage maps were generated and the plasmids used for further characterization. In a parallel study, four clones were isolated from a λ gt11 library of *H. pylori* 84-183 DNA by the same methodology, and the DNA 35 insert from one of four positive clones was amplified by polymerase chain reaction (PCR) using primers based on the known flanking λ gt11 sequences. Recombinant phage DNA

from four positive plaques was purified, and each contained a 0.6 kb insert. Immunoblot analysis of lysates from two clones (λ YB1 and λ YB2) showed similar sized 130 kDa bands that reacted with the adsorbed human antiserum.

- 5 To determine whether the 130 kDa protein was synthesized by a recombinant phage as a fusion protein, cell lysate prepared from λ YB1 was subjected to immunoblot analysis using β -galactosidase specific antiserum. The cross-reactivity shown indicates that the recombinant clone λ YB1
- 10 contains a fusion of the λ gt11 β -galactosidase large (116 kDa) fragment and an H. pylori open reading frame. We cloned the λ YB1 insert into pUC19, but the recombinant (pYB1) did not express any protein.

15 **Gel electrophoresis and immunoblot analysis.**

Lysates from E. coli carrying recombinant lambda gt11, λ ZapII or pBluescript were analyzed by SDS-PAGE and immunoblotting with adsorbed human serum. Discontinuous sodium dodecyl sulfate (SDS)-poly-acrylamide gel electrophoresis (PAGE) was performed as described previously (Blaser et al. Infect. Immun., 42:276-284, 1983) by using a 4.5% stacking gel and a 7.0% separating gel. Samples containing 3 μ g of protein were applied to each gel lane. After electrophoresis, gels were fixed and proteins were resolved by the modified silver stain method of Oakley et al. (Anal. Biochem. 105:361-363, 1980). Concentrated culture supernatants containing protein were diluted in sample buffer and were layered onto the surface of a polyacrylamide gel in a Mini-PROTEAN II slab cell (Bio-Rad Laboratories, Richmond, Calif.). Following electrophoresis, proteins were transferred to nitrocellulose paper by electro blotting for 1 h at 1 amp. After nonspecific binding was blocked, the nitrocellulose paper was incubated at room temperature for 1 h with 1:100 dilutions of serum samples. Alkaline-phosphatase conjugates of goat anti-human IgG, (Tago, Inc.,

Burlingame, Calif.), in a dilution of 1:2,000 were used as the second antibody.

Southern hybridization.

5 H. pylori or C. jejuni chromosomal DNA was digested with either HindIII or EcoRI and BamHI and the resulting fragments were electrophoresed on a 0.7% agarose gel in 0.04 M Tris-acetate -2 mM EDTA buffer (pH 8.2). All hybridization conditions and procedures were exactly
10 as described (Sambrook et al, 1989). Probes were radiolabeled by primer extension using random hexamers (Feinberg and Bogelstein Anal. Biochem., 132:6-13, 1983). Hybridization was carried out at 68°C for 16h in buffer containing 6X SSC (1X SSC is 0.15M NaCl, 0.015M sodium
15 citrate), 0.5% sodium dodecyl sulfate (SDS), 5X Denhardt's solution (1X Denhardt's solution is 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin), and 100 µg/ml salmon sperm DNA. The blots were washed at 60°C in 0.5X SSC and exposed to XAR-2 X-ray film (Eastman Kodak,
20 Rochester, NY).

Colony hybridization.

H. pylori strains were grown on trypticase soy blood agar plates (BBL) and replica copies of these
25 colonies were transferred to nitrocellulose filters. Each filter was placed on 3 mm Whatman paper saturated with 0.2 M NaOH/1.5M NaCl. After 3 min the filter was transferred to 3 mm Whatman paper saturated with 0.4M Tris-Cl (pH 7.6)/2X SSC for 3 min, and then to 2X SSC for 3 min. The
30 colony blot filters were dried in a vacuum oven for 90 min at 80°C and hybridized with radiolabeled pMC3 as described (Sambrook et al, 1989).

Cytotoxin production.

35 H. pylori broth culture supernatants were concentrated 30-fold by ultrafiltration, passaged through a 0.2 µM filter, and incubated with HeLa cells. Briefly,

H. pylori strains were cultured at 37°C in brucella broth (BBL, Microbiology Systems, Cockeysville, MD.) containing 5% defined fetal bovine serum (Hyclone, Logan, Utah), supplemented with 10 mM ammonium chloride to potentiate 5 cytotoxin activity. Broth cultures were incubated in a microaerobic atmosphere on a gyratory shaker at 100 rpm for 72 h. Cultures were centrifuged at 3,000 x g for 15 min, and the cell-free supernatants were stored at -70°C. After thawing, supernatants were concentrated 30-fold by 10 using a 30-kDa ultrafiltration membrane, and retentates were sterilized by passage through a 0.22-μm-pore-size filter. These concentrated culture supernatants (CCS_s) were incubated with HeLa cells (obtained from Allison O'Brien, Uniformed Services University of the Health 15 Sciences, Bethesda, Md.) in twofold dilutions from 1:10 to 1:320 as described previously (Leunk et al. J. Med. Microbiol. 26:93-99, 1988), except that toxicity assays were performed in a total volume of 100 μl in 96-well microtiter plates (Falcon; Becton Dickinson and Co., 20 Lincoln Park, N.J.).

Vacuolation of HeLa cells was quantitated using a neutral red uptake assay. Briefly, a stock solution of 0.5% purified grade neutral red (Sigma Chemical Co., St. Louis, Mo.) was prepared in 0.9% saline and filtered with Whatman no. 1 filter paper. Staining solutions were prepared before each experiment by diluting the stock solution 1:10 in Eagle medium containing 10% fetal bovine serum. After incubation with test samples for 24 h the 25 medium overlaying HeLa cells was removed and replaced with 100 μl of staining solution per well for 4 min. The cells were washed twice with 150 μl of 0.9% saline per well, and the neutral red was extracted from cells by the addition of 100 μl of acidified alcohol per well (Montefiori et al. 30 J. Clin. Microbiol. 26:231-235, 1988). The optical density (OD) at 540 nm of wells was determined by using an 35 MR700 enzyme-linked immunosorbent assay reader (Dynatech,

Alexandria, VA.). All assays were performed in triplicate. In all experiments, the mean OD of wells containing cells incubated with medium alone was less than 0.130 (mean, 0.101 ± 0.007); this background OD was 5 subtracted from the OD of experimental wells to yield a net OD. Of the 32 *H. pylori* strains tested, 15 produced the vacuolating cytotoxin, as determined in this assay (Table 3).

10 Mapping the pBluescript inserts.

After digestion with EcoRI, plasmids pMC1, pMC2, and pMC3 were found to contain DNA inserts of approximately 2.5, 2.7, and 3.6 kb, respectively.

Analysis of restriction endonuclease treatment of the 15 recombinant plasmids identified a conserved 1.2 kb HindIII-digestion fragment in all three (Figure 1). As such, further studies concentrated on pMC3, which contained the largest insert. Analysis of deletion mutations produced by exonuclease III digestion, 20 identified the orientation and approximate location of the open reading frame (ORF) (Figure 1, large arrow).

Sequence analysis of pMC3 and pYB1.

To determine the sequence of the 3.6 kb insert 25 in pMC3, a series of nested ordered deletions of the plasmid using exonuclease III (Figure 1) was performed, as described (Sambrook et al., 1989). In total, the sequence for the entire pMC3 insert representing 3648 bp was determined on both strands (SEQ ID NO:1). The nucleotides 30 are numbered on the right of each line. The nucleotides encoding the glycine at residue number 859 of SEQ ID NO:1 are an artifact of the cloning process and are not a part of the tagA gene. SEQ ID NO:2 provides the deduced amino acid sequence of the nucleic acid shown in SEQ ID NO:1.

35

A long open reading frame commencing at nucleotide 1072 continues to the termination of the

insert. Two other open reading frames in the opposite orientation begin at 645 bp and 264 bp. The deduced amino acids are shown beneath the nucleotides. Potential ribosomal binding-sites (Shine-Delgarno sequence; SD), and 5 putative promoter elements (-35 and -10 sequences) are indicated. Only a single ORF exceeding 300 bases was found in any of the six possible reading frames. This ORF encodes a tagA antigen of 859 amino acids, yielding a predicted protein with a molecular weight of 96,022 10 (SEQ ID NO: 2). The direction of transcription deduced from this ORF is also in agreement with that determined previously by the use of the deletion mutants. However, there is no translation termination signal, indicating that the ORF in pMC3 is truncated. The truncated fragment 15 is rich in basic amino acids (Table 2) and the predicted isoelectric point is 8.0. A potential ribosomal binding site (AGGAG) ends 6 bp upstream of the ORF. The sequence 112 bp upstream of the translational start site exhibits the promoter sequence TATAGT (SEQ ID NO: 1) which 20 resembles the Pribnow consensus promoter sequence TATNATN (Hawley and McClure). This putative -10 region, which is similar to a sigma-70 promoter, is associated with a -35 region, ATGCCA, which shares 4 of 6 bases with the corresponding consensus sequence, TTGACA (Hawley and 25 McClure, 1983). The deduced amino acid composition of the truncated polypeptide is shown in Table 2.

Two smaller ORFs, each proceeding in the opposite direction, also were identified (SEQ ID NO: 1). 30 The first, encoding a polypeptide of 79 amino acids, commences at bp 645 and is not proceeded by an obvious Shine Delgarno or putative promoter sequence. The second ORF commences at bp 264 and encodes 88 amino acids before the end of the insert. This truncated ORF is preceded by 35 a Shine Dalgarno sequence, and the sequence TTTGAT 90 bp upstream of the translational start site resembles the -10 consensus promoter site, followed by the sequence TTGTCA,

which shares 5 of 6 bases with the -35 consensus sequence (Hawley and McClure, 1983).

The 0.6 kb insert in pYB1 was sequenced using both
5 forward and reverse primers of the known λgt11 flanking
sequences along with additional primers based on
experimentally-derived insert sequences. The first 464
bases of the 620 bp pYB1 sequence overlapped with the end
of pMC3, but the ORF still continued.

10

Serologic recognition of the truncated recombinant tagA antigen.

In addition to the index case, sera from H. pylori-infected persons that recognize the tagA antigen
15 from H. pylori strain 84-183 recognize the recombinant polypeptide. For this analysis, we studied serum from 6 persons not infected with H. pylori, and from 14 infected persons (7 did and 7 did not recognize the 120-128 kDa
20 antigen from strain 84-183). Using lysates of E. coli XL1-Blue transformed with pMC3 and immunoblotting, there is clear recognition of the 96 kDa antigen by human serum IgG. In total, 4 of 7 sera that recognize the native 120-128 kDa band also strongly recognize the recombinant
25 protein versus none of the 13 sera tested that do not recognize the 120-128 kDa band ($p=0.007$, Fisher's exact test, 2-tailed). If weak reactions to the pMC3 band are considered, then all 7 sera that recognize the 120-128 kDa band, and 3 of 13 of the non-recognizing sera react to the
30 recombinant protein ($p=0.003$, Fisher's exact test, 2-tailed). Thus, the recombinant protein produced by pMC3 can be used for serologic assays to detect antibodies to the H. pylori 120-128 kDa antigen.

35 **Conservation of the tagA gene.**

To determine whether other H. pylori strains possess the tagA gene or homologous sequences, 32 strains were studied by colony hybridization using pMC3 as a probe

(Table 3). A positive signal was obtained from 19 (59.3%) of these strains. SDS-PAGE and immunoblotting of whole cells of these strains indicated that 19 (59.3%) of the 32 strains expressed a band at 120-128 kDa. The immunoblot and colony hybridization findings correlated completely; all 19 *H. pylori* strains expressing the protein possessed a gene homolog, in comparison to none of the 13 strains not expressing the protein ($p<0.001$, Fisher's exact test, one-tailed). In addition, all 15 strains producing the vacuolating cytotoxin showed both pMC3 hybridization and presence of the 120-128 kDa band (Table 3).

To gain information on the restriction fragment polymorphism of the tagA gene and whether there are multiple homologous genes in each bacterial genome, genomic DNA from 4 *H. pylori* strains was prepared and Southern hybridization performed using pMC3 as the probe. Two strains expressing the 120-128 kDa protein and with positive colony hybridization now showed strong hybridization to a HindIII restriction fragment migrating at approximately 1.2 kb, and weaker bands at 3.0 and 3.3 kb. For a third strain that showed the phenotype and had a positive colony hybridization, the probe hybridized strongly in the Southern analysis to a band of about 1.1 kb; no weaker bands were seen. A band migrating at less than 0.5 kb that hybridized weakly with the probe was present in all three strains. An *H. pylori* strain that expressed no 120-128 kDa protein and that had a negative colony hybridization, as well as a *C. jejuni* strain used as a control, showed lack of hybridization in the Southern analysis. Hybridization of pMC3 to chromosomal DNA from strains 84-183 and 60190 digested with EcoRI and BamHI also showed polymorphism, confirming the heterogeneity observed with the other restriction enzyme. These studies indicate that although homologs of tagA exist in other *H. pylori* strains, there is heterogeneity in either intragenic or flanking sequences.

The present example provides a cloned fragment of H. pylori genomic DNA that includes the majority of a gene that encodes an important H. pylori antigen. The evidence that pMC3 contains the gene encoding the tagA antigen may be summarized as follows: (i) neither the protein nor the gene are present in all H. pylori strains; (ii) only strains expressing the 120-128 kDa protein hybridize with pMC3 and strains that do not express the protein do not hybridize; (iii) sera from H. pylori-infected persons that recognize the 120-128 kDa antigen recognize the recombinant tagA product significantly more frequently than do control sera.

The partial sequences of tagA and the two other ORFs have no identity with the N-terminus or 3 internal sequences from the 87 kDa cytotoxin. This finding is consistent with earlier observations that the 120-128 kDa and 87 kDa proteins are antigenically unrelated (Cover and Blaser J. Biol. Chem., 1992). Comparison of the truncated deduced gene product revealed little direct homology with known proteins.

The tagA gene or homologous genes are present in approximately 60% of the H. pylori isolates studied but absent from the others. As indicated by the Southern analysis, there is evidence for restriction fragment polymorphism even when only a small number of strains are examined. Absence of a homolog correlated exactly with lack of expression of an antigenic band at 120-128 kDa. Thus, the phenotype lacking this band is not due to deficiencies related to transcription or expression but rather to the absence of the implicated gene.

The presence of genomic DNA containing at least the truncated tagA gene is highly associated with cytotoxin production. A minority of strains that possess the tagA gene do not produce detectable levels of

cytotoxin. This phenomenon may reflect suboptimal sensitivity in the cell culture assay to detect toxin, or may indicate that factors other than the tagA antigen are associated with toxin activity.

5

As shown by the immunoblot studies, the pMC3 products are excellent diagnostic reagents for detection of human serum antibodies to the tagA antigen. Use of this recombinant protein can readily supply sufficient

10 antigen to aid in development of immunoassays to determine which persons are infected with H. pylori strains producing the native 120-128 kDa protein, and heterologous antibodies raised against the pMC3 gene product can be used to determine which strains produce the tagA antigen.

15 Knowledge of the DNA sequence of pMC3 permits the construction of oligonucleotides for use as hybridization probes or for primers for PCR. Such techniques are also used for rapid detection of infection due to a strain with the implicated genotype. Creation of deletion mutants

20 enables elucidation of the role of this gene product and provides both therapeutic reagents and vaccine candidates. Such diagnostic methods and mutants are detailed herein.

Table 1
Helicobacter pylori strains used in this study

<u>Strain designation</u>	<u>Isolation locale</u>	<u>Expression of 120-128 kDa antigen^a</u>	<u>Expression of vacuolating cytotoxin activity^b</u>
Tx30a	Texas	-	-
84-183	Texas	+	+
60190	England	+	+
87-29	Colorado	+	+
86-313	Colorado	-	-
87-199	Colorado	+	+
86-385	Colorado	-	-
87-33	Colorado	+	+
87-81	Colorado	+	+
87-91	Colorado	+	+
87-90	Colorado	-	-
87-226	Colorado	+	-
87-225	Colorado	-	-
87-230	Colorado	-	-
87-75	Colorado	-	-
87-203	Colorado	-	-
87-6	Colorado	+	-
86-338	Colorado	-	-
86-63	New York	+	-
86-86	New York	+	+
86-332	Minnesota	+	+
92-18	Tennessee	+	+
92-19	Tennessee	+	+
92-20	Tennessee	-	-
92-21	Tennessee	+	+
92-22	Tennessee	+	-
92-23	Tennessee	-	-

<u>Strain designation</u>	<u>Isolation locale</u>	<u>Expression of 120-128 kDa antigen^a</u>	<u>Expression of vacuolating cytotoxin activity^b</u>
92-24	Tennessee	-	-
92-25	Tennessee	+	+
92-26	Tennessee	+	+
92-27	Tennessee	+	+
92-28	Tennessee	-	-

^a Recognition of 120-128 kDa band in cell lysates by human serum as detected by immunoblot (Cover et al., 1990).

^b Production of vacuolating cytotoxin as detected in HeLa cell culture (Cover et al. *Infect. Immun.* 59:1264-1270, 1991).

Table 2

Amino acid composition of truncated 859 amino acid tagA polypeptide as deduced from pMC3

<u>Amino acid</u>	<u>Number of residues</u>	<u>Percent of 859 amino acids</u>
Ala	60	7.0
Cys	2	0.2
Asp	62	7.2
Asn	82	9.5
Glu	59	6.9
Gln	48	5.6
Phe	44	5.1
Gly	54	6.3
His	12	1.4
Ile	50	5.8
Lys	101	11.8
Leu	67	7.8
Met	12	1.4
Pro	24	2.8
Arg	22	2.6
Ser	63	7.3
Thr	30	3.5
Val	47	5.5
Trp	4	0.4
Tyr	16	1.9

Table 3

Correlation between presence of 120-128 kDa band by immunoblot, hybridization with pMC3, and cytotoxin production by 32 *H. pylori* isolates from humans

Strain characteristics				
Presence of 120-128 kDa band on immunoblot^a	Hybridization of pMC3 to <i>H. pylori</i> colony^b	Cytotoxin production in cell culture assay^c		Number of strains
+	+	+		15
-	-	-		13
+	+	-		4
-	-	+		0
-	+	-		0
+	-	-		0
+	-	+		0
-	+	+		0

^a Recognition of 120-128 kDa band in cell lysates by human serum as detected by immunoblot (Cover et al., 1990).

^b Hybridization of pMC3 to lysed *H. pylori* cells in colony blot (Sambrook et al., 1989).

^c Production of vacuolating cytotoxin as detected in HeLa cell culture (Cover et al., 1991).

EXAMPLE 2

**Construction and characterization of a tagA-negative
strain of Helicobacter pylori**

5

Bacterial strains, vectors and growth conditions.

H. pylori strain 84-183 (ATCC 53726) used in this study was from the culture collection of the 10 Vanderbilt University Campylobacter/Helicobacter Laboratory and was chosen because it has been extensively characterized. Stock cultures were maintained at -70°C in Brucella broth (BBL Microbiology Systems, Cockeysville, MD) supplemented with 15% glycerol. H. pylori strains were 15 grown in Brucella broth supplemented with 5% fetal bovine serum or on blood agar plates supplemented with nalidixic acid (50 mg/liter), vancomycin (10 mg/liter), polymyxin B (5000 U/liter), and trimethoprim (5 mg/liter) under microaerobic conditions at 37°C for 48 hours. E. coli 20 strain DH5 α (Stratagene, La Jolla, CA) used for transformation, was grown in LB medium. As described above, pMC3 contains the truncated tagA gene on a 3.5 kb insert in pBluescript. Plasmid pILL600 (Labigne-Roussel et al. J. Bacteriol., 170:1704, 1988) was used as a source 25 of a C. coli kanamycin (km) resistance gene.

Chemicals and enzymes.

Final concentrations of ampicillin (100 μ g/ml) and kanamycin (50 μ g/ml) were used whenever necessary.

30 Restriction enzymes, T4 DNA ligase, E. coli DNA polymerase large (Klenow) fragment were from Promega and United States Biochemicals (Cleveland, OH). α -³²P-dATP (650 Ci/mmol) was from ICN Radiochemicals (Irvine, CA).

35 Genetic techniques.

Chromosomal DNA was prepared as described above. Plasmids were isolated by the procedure of Birnboim and Doly (1979). All other standard molecular genetic techniques were performed as described (Sambrook et al.,

1989). DNA fragments used as probes for hybridization experiments were gel-purified.

Introduction of km cassette into H. pylori strain 84-183.

5 An E. coli kanamycin-resistance gene was inserted into the unique NdeI site of pMC3 to create pMC3:km (Figure 2). This construct was introduced directly into H. pylori strain 84-183 by electroporation. Briefly, H. pylori cells grown on blood agar plates for 48
10 h were harvested, washed three times in electroporation buffer (15% glycerol/5% sucrose) and suspended in 200 μ l of the buffer. Plasmid DNA from pMC3:km was isolated by a rapid (mini-prep) alkaline-lysis method of Birnboim and Doly and was added to the cells and incubated for 5 min on
15 ice. The cells and DNA were transferred to 0.2 cm electroporation cuvette in a Gene-pulsar apparatus (Bio-Rad), and high voltage pulses (25F, 2.5 kv and 200 Ω) were delivered as described previously (Ferrero et al. J. Bacteriol., 174:4212, 1992). Following electroporation,
20 the cells were suspended in 400 μ l of LB media and spread on blood agar plates. The plates were incubated at 37°C under microaerobic conditions for 24 h, then cells were harvested, plated on blood agar plates containing 50 μ g/ml of kanamycin, and incubated microaerobically for 48 h.

25

The cloning vector used was unable to replicate in H. pylori and selection on kanamycin-containing media yielded kanamycin-resistant recombinants. From approximately 10^{10} H. pylori cfu, 3000 transformants (10^7)
30 were obtained when 500 ng of plasmid DNA was used.

Colony hybridization.

Fifty kanamycin-resistant transformants obtained by electroporation were grown on blood agar plates and
35 replica copies of these colonies were transferred to nitrocellulose filters. Each filter was placed on 3 mM Whatman paper saturated with 0.2 M NaOH/1.5 M NaCl. After

3 min the filter was transferred to 3 mM Whatman paper, saturated with 0.4 M Tris-HCl (pH 7.6)/2 X SSC for 3 min, and then to 2 X SSC for 3 min. The colony blot filters were dried in a vacuum oven for 90 min at 80°C and 5 hybridized with radiolabeled pBluescript or the km-resistance gene, as described above. The colony blots were washed at 60°C in 0.5X SSC and exposed to XAR-2 X-Ray film (Eastman Kodak, Rochester, NY).

10 **Gel electrophoresis and immunoblot analysis.**

Lysates of E. coli carrying pBluescript, pMC3 or pMC3:km or of H. pylori cells were prepared and analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Immunoblotting of whole cell extracts derived from wild-type and mutants 1, 21 and 22 was performed as detailed above using a 1:300 dilution of adsorbed human sera, and a 1:2000 dilution of goat anti-human immunoglobulin alkaline phosphatase conjugate as the secondary antibody, as described above. These studies showed that isogenic 15 mutant strains 1, 21, and 22 have no antigenic tagA gene product.

Southern hybridizations.

Southern hybridization of wild-type H. pylori 25 strain 84-183 and kanamycin-resistant transformants M21 and M22 were performed. H. pylori chromosomal DNA was digested with HindIII or BamHI and SacI and the resulting fragments were electrophoresed on a 0.7% agarose gel and transferred to nylon membrane. Probes were gel-purified 30 DNA fragments derived from pMC4 or pILL600 and were radiolabeled by primer extension using random hexameric oligonucleotides as described above. The DNA was then transferred to a nylon membrane and hybridized with ³²P-labeled pMC4 or the 1.3 kb km cassette under conditions of 35 high stringency. Hybridizations were performed in a solution of 6X SSC, 0.5% SDS, 5X Denhardt's solution and

100 µg/ml salmon sperm DNA and the blots were washed for 30 min. at 60°C in 0.5X SSC/0.1% SDS.

Genotypic characterization of the transformants.

5 To provide genetic evidence that the tagA gene is disrupted in the transformant strains, DNA isolated from wild-type strain 84-183 and *H. pylori* mutants 21 and 22 was digested with the restriction endonuclease HindIII or BamHI and SacI. After separation of the digested DNA
10 on an agarose gel the DNA was transferred to a nylon membrane and hybridized to pMC4 which is a tagA probe. This probe hybridized to approximately 20 and 1.0 kb BamHI-SacI fragments in the wild-type strain, whereas the 1.0 kb BamHI-SacI fragment is lost and a new 2.3 kb
15 hybridizing fragment was observed in both mutant strains without disruption of the other bands. Similarly, a 1.2 kb HindIII fragment was lost and a 2.2 kb fragment gained in both mutants because of the kanamycin resistance gene insertion. The kanamycin gene probe hybridized only with
20 the 2.3 kb BamHI-SacI and 2.2 kb HindIII fragment in mutants 21 and 22 strains, which indicate that replacement had occurred in the tagA gene. Thus, the tagA gene in strain 84-183 had been mutagenized by insertion of the km gene.

25

Cytotoxin production.

Cytotoxin production was assayed as described above and the results shown in Table 4. The results indicate that neither the intact tagA antigen nor the
30 intact tagA gene is required for vacuolation.

Table 4

**Cytotoxin production by wild-type *H. pylori* strains
and tagA⁻ mutants**

Supernatant dilution	Optical density ^a			
	84-183	M1	M22	87-203 ^b
1:5	0.21 ± 0.04	0.26 ± 0.04	0.23 ± 0.05	0 ± 0.02
1:10	0.16 ± 0.02	0.20 ± 0.03	0.13 ± 0.02	0.01 ± 0.01
1:20	0.13 ± 0.01	0.15 ± 0.02	0.10 ± 0.02	0.01 ± 0.01
1:40	0.09 ± 0.02	0.06 ± 0.01	0.06 ± 0.02	0.01 ± 0
1:80	0.03 ± 0.01	0.04 ± 0	0.02 ± 0.01	0.01 ± 0
1:160	-0.01 ± 0.02	-0.01 ± 0.01	-0.01 ± 0.02	-0.02 ± 0.02

^a Net optical density as measured in neutral red assay of cytotoxin-induced vacuolation, as described above (Cover et al., 1991).

^b Strain 87-203 is a strain known not to produce cytotoxin.

EXAMPLE 3

Full Length tagA Gene and Gene Product

5 Cloning and sequencing of the full length gene.

To isolate the full-length gene, we next used the 0.6 kb fragment of pYB1 as a probe to screen the λZapII library of *H. pylori* 84-183. Five positive plaques were purified and the pBluescript plasmids containing the cloned DNA inserts were excised by co-infection with the helper phage. Each of the five positive clones contained DNA inserts of 2 to 3 kb (data not shown). The clone designated pYB2, which contains a 2.7 kb insert, was chosen for further study and a restriction map generated (Figure 3). A series of nested deletions starting at

either end of the 2.7 kb insert of pYB2 was performed using exonuclease III. Using overlapping deletion clones of pYB2, we determined 1969 bp sequence in both strands. As expected, the first 785 bases of this sequence (SEQ ID 5 NO:3, beginning with nucleotide 2864) overlapped with the end of pMC3. Translation of the complete nucleotide sequence generated from pMC3, pYB1 and pYB2 in all possible reading frames revealed a single open reading frame of 3,543 nucleotides initiated by an ATG codon at 10 position 1072 and terminated by a TAA codon at position 4,615. The sequence encodes a protein of 1181 amino acid residues (SEQ ID NO:4) and the calculated molecular weight of the deduced polypeptide is 131,517 daltons. A sequence that could form a potential stem-loop structure in the 15 mRNA and which could serve as a transcription termination site ($\Delta G = -14.4$ Kcal) extends from nucleotides 4642 to 4674 (SEQ ID NO:3).

Homologies of the tagA polypeptide with other proteins.

20 Search of Swiss.Prot version 21, and NBRF-PIR protein data banks showed no striking homologies with the full length tagA antigen (SEQ ID NO:4). However, among the homologies with the highest scores were chloroplast H⁺-transporting ATP synthases (Hiratsuka et al. Mol. Gen. 25 Genet. 217:185-194, 1989; Rodermel and Bogorad Genetics, 116:127-139, 1987), and a sodium channel protein (Trimmer, et al. Neuron 3:33-49, 1989) with 16.8% and 17.3% identity, and 50% and 42.6% conserved amino acids in the region between residues 1-482 and 123-1182, respectively. 30 No significant homologies were observed when the amino acid sequences of the other two ORFs contained in pMC3 were compared with the protein data bases.

The content of basic amino acids [141 lysines, 35 (11.9%) and 117 asparagines (9.9%)] in the tagA product was unusually high and was consistent with the predicted isoelectric point of the peptide (8.89). A hydropathicity

plot indicated that the deduced protein is predominantly hydrophilic. An interesting feature of the primary structure of this protein is the presence of structures of homopolymeric amino acid sequence, most notably

5 polyasparagine (SEQ ID NO:4, Position 3705). In searches comparing this asparagine-rich region with various protein sequence data bases, there was strong homology with sequences from yeast (Forsburg and Guarente Genes Dev. 3:1166-1178, 1989; Hudspeth et al. Cell 30:617-626, 1982;

10 Ju et al. Mol. Cell. Biol. 10:5226-5234, 1990; O'Hara et al. Nucleic Acids Res. 16:10133-10170, 1988; Rhode et al. Genes Dev. 3:1926-1939, 1989; Tanaka et al. Mol. Cell. Biol. 9:757-768, 1989; and Toda et al. Genes Dev. 2:517-527, 1988) and Plasmodium (Stahl et al. Nucleic Acids Res. 14:3089-3102, 1986) nucleotide-binding proteins.

Polyasparagine is also found in the DNA-binding regulatory product of the lac9 gene of Kluyveromyces var.lactis (Salmeron and Johnston Nucleic Acids Res. 14:7767-7781, 1986) and potassium transport protein (TRK1) of

15 20 Saccharomyces cerevisiae (Gaber et al. Mol. Cell. Biol. 8:2848-2859, 1989).

Construction of the full length tagA gene.

To construct the full length tagA gene, we utilized

25 the unique SacI restriction site located in both pMC3 and pYB2 (Figure 3). First, the 3.6 kb tagA fragment of pMC3 was cloned into a pUC19 vector under the control of the lacZ promoter, to generate pUT1. Next, the 2.6 kb SacI fragment from pYB2 was cloned into sacI-digested pUT1. A

30 clone with the correct orientation was selected, which was named pUT2. An identical clone (pEM3), but present in the pGEM3z vector, has been deposited with the ATCC in compliance with the requirements of the Budapest Treaty under Accession No. 69273. E. coli cells containing pUT2

35 or pEM3 expressed the immunoreactive H. pylori 128 kDa protein.

Detection of human serologic responses to the recombinant tagA protein by Western blotting.

To determine whether human sera reacted with the full-length recombinant tagA protein, lysate from pEM3-
5 containing cells was electrophoresed on a 7% acrylamide gel, and electroblotted onto nitrocellulose paper. Sera from 10 H. pylori infected humans and 10 uninfected humans were diluted 1:100 and tested for reactivity with the recombinant protein. Sera from 7 H. pylori infected
10 persons recognized the tagA protein, compared to sera from 1 of 10 uninfected persons ($p=0.01$, one-tailed Fisher's exact test). Thus, the recombinant full-length protein was a useful antigen for assessing human responses to H. pylori.

15

EXAMPLE 4

DNA probe for detecting tagA gene sequences.

20 Helicobacter pylori strains were collected from 66 patients: duodenal ulceration was present in 18 patients, gastric ulceration was present in 15 patients, and 33 patients had no ulceration. To determine whether the tagA gene was present in the strains isolated from patients
25 with peptic ulceration more frequently than in strains isolated from patients with gastritis only, each of the 66 strains were colony blotted using radiolabeled pMC3 as a probe. Briefly, H. pylori strains were grown on Trypticase soy blood agar plates (BBL), and replica copies
30 of the colonies were transferred to nitrocellulose filters. Each filter was placed on Whatman 3MM paper saturated with 0.2 M NaOH-1.5 M NaCl. After 3 min, the filter was transferred to Whatman 3MM paper saturated with 0.4 M Tris-Cl (pH 7.6)-2x SSC for 3 min and then to 2x SSC
35 for 3 min. Colony blot filters were dried in a vacuum oven for 90 min at 80°C and hybridized with radiolabeled pMC3. This assay detected tagA genetic sequences in 15

(83%) of the 18 patients with duodenal ulcer, 11 (73%) of the 15 patients with gastric ulcer, and 16 (51%) of the 33 patients with no ulceration. Thus, tagA sequences were detected in Helicobacter pylori strains from patients with 5 peptic ulceration significantly more frequently than in patients without ulceration ($p=0.02$).

EXAMPLE 5

10

ELISA for detection of serum antibodies to recombinant tagA

E. coli XL1Blue containing pEM3 (which encodes the 15 entire tagA gene) was cultured in 2XYT medium containing 100 ug/ml carbenicillin. After centrifugation of the culture, cells were suspended in 20 mM Tris (pH 8.0) containing 10 mM EDTA and 0.1 mM dithiothreitol, and sonicated. The sonicated preparation was centrifuged, and 20 the pellet was washed for 30 minuets with 20 mM Tris (pH 8.0) containing 1M urea, 10 mM EDTA, and 0.1 mM dithiothreitol. After centrifugation, the pellet was suspended in the same buffer containing 6 M urea for 30 minutes. This preparation was centrifuged, and the tagA- 25 containing supernatant was purified further by two liquid chromatography steps. First, the preparation was applied to a MonoQ column (Pharmacia) in 60 mM Tris (pH 7.6) containing 6 M urea, and the tagA peak was eluted with a gradient of the same buffer containing 1M NaCl. This 30 fraction was then applied to a Superose 6 column in 50 mM Tris (pH 7.6) containing 0.1 M NaCl and 1% SDS. This procedure resulted in the purification of a fraction which contained recombinant TagA (approximately 130 kDa in size) as the predominant antigen, as assessed by Western 35 blotting with human serum.

The purified recombinant tagA was then used as the antigen in an ELISA (1 µg/well in 96-well plates). Human sera was obtained from 6 patients infected with tagA+ Helicobacter pylori strains, 6 patients infected with 5 tagA- strains, and 6 patients who were not infected with Helicobacter pylori. The sera were diluted 1:500 and tested in the ELISA (see table 5). Sera from patients infected with tagA+ strains reacted to a significantly greater extent than sera from patients infected with tagA- 10 strains ($p=0.03$) or sera from uninfected patients ($p=0.03$).

Table 5

15 ELISA reactivity of sera from H. pylori-infected and uninfected patients with recombinant tagA

20	Patient group	<u>H. pylori</u> strain	n	ELISA optical density (mean ± SEM)
	Infected	<u>tagA+</u>	6	0.341±0.13
	Infected	<u>tagA-</u>	6	0.022±0.01
25	Uninfected	None	6	0.009±0.00

Throughout this application various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state 5 of the art to which this invention pertains

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: COVER, TIMOTHY L.
BLASER, MARTIN J.
TUMMURU, MURALI K.R.
- (ii) TITLE OF INVENTION: THE tagA GENE AND METHODS FOR DETECTING PREDISPOSITION TO PEPTIC ULCERATION
- (iii) NUMBER OF SEQUENCES: 4
- (iv) CORRESPONDENCE ADDRESS:
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 - (E) COUNTRY: USA
 - (F) ZIP: 30303
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
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 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: SPRATT, GWENDOLYN D.
 - (B) REGISTRATION NUMBER: 36,016
 - (C) REFERENCE/DOCKET NUMBER: 2200.009
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 404/688-0770
 - (B) TELEFAX: 404/688-9880

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 3648 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Helicobacter pylori

(ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 1072..3648

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATGGGCTGCG CGTAACGAAA AACAGTCGCT TGACCTCTT TGATGTCATC AGAGATTT	60
CAAATATCCG CTATACCTT GACTCCTAGA GCGCAACCAC CTACGATCGC TAGAACAGAA	120
ATGATCTGAA CCACCAAAGT TTTAGTCTCA GTAATGCCTG ATGCAGGACT GTCGAAAGCC	180
ATTAAGGAT TGGCTGCTAT CGCTAGCCCT AAAGTTACTA CAACTTCCTT GTAGCTGTCA	240
GTGATTCTTG TAAAAAATT CATGCGTTTC CTTCAAATT GAAATCAATC GTTTGAGTAT	300
ATCAAAAAAA AGTATTTTA TACTATTCA ACAAGCGCTA CTTTATAATT TAAATCAAAA	360
CCGACGCTTT TGTTTGACAA CTGATATAAT TTAGGAACAA TAAACCTACT TGTCCAACC	420
ATTTTCTTT CTCAGTCAT CGTAGAATTG TAGATCTTTA GGATCTTGA TGTATTTTT	480
AATCGTCTCA GGTTGAAACC TAAAAACAAG CAGAAACAAA CCCAAGCTGA TCAGAGTGAG	540
AATAAGCTC CATTAAAGC AACTCCATAA ACCACTAAAG AAACTTTTTG TGAGACTCTC	600
TTGAAAATC TGT CCTATTG ATTGTTTTC CATTGTTT CCCATGCGGA TCACAAACGC	660
TTAATTACAA ATACATACTA TAATAAGTAT GGCACACACA AACCAAACCA TTTTTAGAAC	720
GCTTCATGCA CTCACCTTGC TCCTAACCAT TTCTCCAACC ATCTTAGCG TTGCATTGA	780
TTTCTCAAA AAGGCTCATT TCTTAGTTTC TTTATTCTT AAAATTTTC CATTCTAGCA	840
AATTTTGTT AATTGTGGGT AAAATGTGA ATCGTTCTA GCTTTAGAC GCTTGCAACG	900
ATCGGACTTT TTTCAATATT AATGAAAAAA TGCCAAATAT TCTAAATATT GTGGTATAGT	960
GATAACGTTA AAAGACACGA ATTGCATACT CAAAGTGTGT AGTAGTTTT AGCGGTCTT	1020
GATACCAATA AGATACCGAT AGGTATGAAA CTAGGTATAG AAGGAGAAAC A ATG ACT	1077
Met Thr	
1	
AAC GAA ACT ATT GAC CAA CAA CCA CAA ACC GAA GCG GCT TTT AAC CCG	1125
Asn Glu Thr Ile Asp Gln Gln Pro Gln Thr Glu Ala Ala Phe Asn Pro	
5	10
15	
CAG CAA TTT ATC AAT AAT CTT CAA GTA GCT TTT CTT AAA GTT GAT AAC	1173
Gln Gln Phe Ile Asn Asn Leu Gln Val Ala Phe Leu Lys Val Asp Asn	
20	25
30	
GCT GTC GCT TCA TAC GAT CCT GAT CAA AAA CCA ATC GTT GAT AAG AAC	1221
Ala Val Ala Ser Tyr Asp Pro Asp Gln Lys Pro Ile Val Asp Lys Asn	
35	40
45	50

55

GAT AGG GAT AAC AGG CAA GCT TTT GAG GGA ATC TCG CAA TTA AGG GAA Asp Arg Asp Asn Arg Gln Ala Phe Glu Gly Ile Ser Gln Leu Arg Glu 55 60 65	1269
GAA TAC TCC AAT AAA GCG ATC AAA AAT CCT ACC AAA AAG AAT CAG TAT Glu Tyr Ser Asn Lys Ala Ile Lys Asn Pro Thr Lys Lys Asn Gln Tyr 70 75 80	1317
TTT TCA GAC TTT ATC AAT AAG AGC AAT GAT TTA ATC AAC AAA GAC AAT Phe Ser Asp Phe Ile Asn Lys Ser Asn Asp Leu Ile Asn Lys Asp Asn 85 90 95	1365
CTC ATT GTC GTG GAA TCT TCC ACA AAG AGC TTT CAG AAA TTT GGG GAT Leu Ile Val Val Glu Ser Ser Thr Lys Ser Phe Gln Lys Phe Gly Asp 100 105 110	1413
CAG CGT TAC CGA ATT TTC ACA AGT TGG GTG TCC CAT CAA AAC GAT CCG Gln Arg Tyr Arg Ile Phe Thr Ser Trp Val Ser His Gln Asn Asp Pro 115 120 125 130	1461
TCT AAA ATC AAC ACC CGA TGC ATC CGA AAT TTT ATG GAA CAT ACC ATA Ser Lys Ile Asn Thr Arg Cys Ile Arg Asn Phe Met Glu His Thr Ile 135 140 145	1509
CAA CCC CCT ATC CCT GAT GAC AAA GAA AAA GCA GAG TTT TTG AAA TCT Gln Pro Pro Ile Pro Asp Asp Lys Glu Lys Ala Glu Phe Leu Lys Ser 150 155 160	1557
GCC AAA CAA TCT TTT GCA GGA ATC ATC ATA GGG AAT CAA ATC CGA ACG Ala Lys Gln Ser Phe Ala Gly Ile Ile Ile Gly Asn Gln Ile Arg Thr 165 170 175	1605
GAT CAA AAA TTC ATG GGC GTG TTT GAT GAA TCC TTG AAA GAA AGG CAA Asp Gln Lys Phe Met Gly Val Phe Asp Glu Ser Leu Lys Glu Arg Gln 180 185 190	1653
GAA GCA GAA AAA AAT GGA GGG CCT ACT GGT GGG GAT TGG TTG GAT ATT Glu Ala Glu Lys Asn Gly Gly Pro Thr Gly Gly Asp Trp Leu Asp Ile 195 200 205 210	1701
TTT TTA TCA TTT ATA TTT GAC AAA AAA CAA TCT TCT GAT GTC AAA GAA Phe Leu Ser Phe Ile Phe Asp Lys Lys Gln Ser Ser Asp Val Lys Glu 215 220 225	1749
GCA ATC AAT CAA GAA CCA CTT CCT CAT GTC CAA CCA GAT ATA GCC ACT Ala Ile Asn Gln Glu Pro Leu Pro His Val Gln Pro Asp Ile Ala Thr 230 235 240	1797
AGC ACC ACT CAC ATA CAA GGC TTA CCG CCT GAA TCT AGG GAT TTG CTT Ser Thr Thr His Ile Gln Gly Leu Pro Pro Glu Ser Arg Asp Leu Leu 245 250 255	1845
GAT GAA AGG GGT AAT TTT TCT AAA TTC ACT CTT GGC GAT ATG GAA ATG Asp Glu Arg Gly Asn Phe Ser Lys Phe Thr Leu Gly Asp Met Glu Met 260 265 270	1893

TTA GAT GTT GAG GGC GTC GCC GAC ATG GAT CCC AAT TAC AAG TTC AAT Leu Asp Val Glu Gly Val Ala Asp Met Asp Pro Asn Tyr Lys Phe Asn 275 280 285 290	1941
CAA TTA TTG ATT CAC AAT AAC ACT CTG TCT TCT GTG TTA ATG GGG AGT Gln Leu Leu Ile His Asn Asn Thr Leu Ser Ser Val Leu Met Gly Ser 295 300 305	1989
CAT GAT GGC ATA GAA CCT GAA AAA GTT TCA TTA TTG TAT GCG GGC AAT His Asp Gly Ile Glu Pro Glu Lys Val Ser Leu Leu Tyr Ala Gly Asn 310 315 320	2037
GGT GGT TTT GGA GCC AAG CAC GAT TGG AAC GCC ACC GTT GGT TAT AAA Gly Gly Phe Gly Ala Lys His Asp Trp Asn Ala Thr Val Gly Tyr Lys 325 330 335	2085
GAC CAA CAA GGT AAC AAT GTG GCT ACA ATA ATT AAT GTG CAT ATG AAA Asp Gln Gln Gly Asn Asn Val Ala Thr Ile Ile Asn Val His Met Lys 340 345 350	2133
AAC GGC AGT GGC TTA GTC ATA GCA GGT GGT GAG AAA GGG ATT AAC AAC Asn Gly Ser Gly Leu Val Ile Ala Gly Gly Glu Lys Gly Ile Asn Asn 355 360 365 370	2181
CCT AGT TTT TAT CTC TAC AAA GAA GAC CAA CTC ACA GGC TCA CAA CGA Pro Ser Phe Tyr Leu Tyr Lys Glu Asp Gln Leu Thr Gly Ser Gln Arg 375 380 385	2229
GCA TTG AGT CAA GAA GAG ATC CAA AAC AAA ATA GAT TTC ATG GAA TTT Ala Leu Ser Gln Glu Ile Gln Asn Lys Ile Asp Phe Met Glu Phe 390 395 400	2277
CTT GCA CAA AAC AAT GCT AAA TTA GAC AGC TTG AGC GAG AAA GAG AAA Leu Ala Gln Asn Asn Ala Lys Leu Asp Ser Leu Ser Glu Lys Glu Lys 405 410 415	2325
GAA AAA TTC CGA AAT GAG ATT AAG GAT TTC CAA AAA GAC TCT AAG CCT Glu Lys Phe Arg Asn Glu Ile Lys Asp Phe Gln Lys Asp Ser Lys Pro 420 425 430	2373
TAT TTA GAC GCC CTA GGG AAT GAT CGT ATT GCT TTT GTT TCT AAA AAA Tyr Leu Asp Ala Leu Gly Asn Asp Arg Ile Ala Phe Val Ser Lys Lys 435 440 445 450	2421
GAC CCA AAA CAT TCA GCT TTA ATT ACT GAG TTT AAT AAG GGG GAT TTG Asp Pro Lys His Ser Ala Leu Ile Thr Glu Phe Asn Lys Gly Asp Leu 455 460 465	2469
AGC TAC ACT CTC AAA GTT ATG GGA AAA AAG CAG ATA AAG GCT TTA GAT Ser Tyr Thr Leu Lys Val Met Gly Lys Lys Gln Ile Lys Ala Leu Asp 470 475 480	2517
AGG GAG AAA AAT GTC ACT CTT CAA GGT AAC CTA AAA CAT GAT GGC GTG Arg Glu Lys Asn Val Thr Leu Gln Gly Asn Leu Lys His Asp Gly Val 485 490 495	2565

ATG TTT GTT AAT TAT TCT AAT TTC AAA TAC ACC AAC GCC TCC AAG AGT Met Phe Val Asn Tyr Ser Asn Phe Lys Tyr Thr Asn Ala Ser Lys Ser 500 505 510	2613
CCC AAT AAG GGT GTA GGC GTT ACG AAT GGC GTT TCC CAT TTA GAA GCA Pro Asn Lys Gly Val Gly Val Thr Asn Gly Val Ser His Leu Glu Ala 515 520 525 530	2661
GGC TTT AGC AAG GTG GCT GTC TTT AAT TTG CCT AAT TTA AAT AAT CTC Gly Phe Ser Lys Val Ala Val Phe Asn Leu Pro Asn Leu Asn Asn Leu 535 540 545	2709
GCT ATC ACT AGT GTC GTA AGG CGG GAT TTA GAG GAT AAA CTA ATC GCT Ala Ile Thr Ser Val Val Arg Arg Asp Leu Glu Asp Lys Leu Ile Ala 550 555 560	2757
AAA GGA TTG TCC CCA CAA GAA GCT AAT AAG CTT GTC AAA GAT TTT TTG Lys Gly Leu Ser Pro Gln Glu Ala Asn Lys Leu Val Lys Asp Phe Leu 565 570 575	2805
AGT AGC AAC AAA GAA TTG GTT GGA AAA GCT TTA AAC TTC AAT AAA GCT Ser Ser Asn Lys Glu Leu Val Gly Lys Ala Leu Asn Phe Asn Lys Ala 580 585 590	2853
GTA GCT GAA GCT AAA AAC ACA GGC AAC TAT GAC GAG GTG AAA CGA GCT Val Ala Glu Ala Lys Asn Thr Gly Asn Tyr Asp Glu Val Lys Arg Ala 595 600 605 610	2901
CAG AAA GAT CTT GAA AAA TCT CTA AAG AAA CGA GAG CAT TTG GAG AAA Gln Lys Asp Leu Glu Lys Ser Leu Lys Lys Arg Glu His Leu Glu Lys 615 620 625	2949
GAT GTA GCG AAA AAT TTG GAG AGC AAA AGC GGC AAC AAA AAT AAA ATG Asp Val Ala Lys Asn Leu Glu Ser Lys Ser Gly Asn Lys Asn Lys Met 630 635 640	2997
GAA GCA AAA GCT CAA GCT AAC AGC CAA AAA GAT GAG ATT TTT GCG TTG Glu Ala Lys Ala Gln Ala Asn Ser Gln Lys Asp Glu Ile Phe Ala Leu 645 650 655	3045
ATC AAT AAA GAG GCT AAT AGA GAC GCA AGA GCA ATC GCT TAC GCT CAA Ile Asn Lys Glu Ala Asn Arg Asp Ala Arg Ala Ile Ala Tyr Ala Gln 660 665 670	3093
AAT CTT AAA GGC ATC AAA AGG GAA TTG TCT GAT AAA CTT GAA AAT ATC Asn Leu Lys Gly Ile Lys Arg Glu Leu Ser Asp Lys Leu Glu Asn Ile 675 680 685 690	3141
AAC AAG GAT TTG AAA GAC TTT AGT AAA TCT TTT GAT GGA TTC AAA AAT Asn Lys Asp Leu Lys Asp Phe Ser Lys Ser Phe Asp Gly Phe Lys Asn 695 700 705	3189
GGC AAA AAT AAG GAT TTC AGC AAG GCA GAA GAA ACG CTA AAA GCC CTT Gly Lys Asn Lys Asp Phe Ser Lys Ala Glu Glu Thr Leu Lys Ala Leu 710 715 720	3237

AAA GGC TCG GTG AAA GAT TTA GGT ATC AAT CCG GAA TGG ATT TCA AAA Lys Gly Ser Val Lys Asp Leu Gly Ile Asn Pro Glu Trp Ile Ser Lys 725 730 735	3285
GTT GAA AAC CTT AAT GCA GCT TTG AAT GAA TTC AAA AAT GGC AAA AAT Val Glu Asn Leu Asn Ala Ala Leu Asn Glu Phe Lys Asn Gly Lys Asn 740 745 750	3333
AAG GAT TTC AGC AAG GTA ACG CAA GCA AAA AGC GAC CAA GAA AAT TCC Lys Asp Phe Ser Lys Val Thr Gln Ala Lys Ser Asp Gln Glu Asn Ser 755 760 765 770	3381
ATT AAA GAT GTG ATC ATC AAT CAA AAG ATA ACG GAT AAA GTT GAT GAA Ile Lys Asp Val Ile Ile Asn Gln Lys Ile Thr Asp Lys Val Asp Glu 775 780 785	3429
CTC AAT CAA GCG GTA TCA GTG GCT AAA ATA GCG TGC GAT TTC AGT GGG Leu Asn Gln Ala Val Ser Val Ala Lys Ile Ala Cys Asp Phe Ser Gly 790 795 800	3477
GTA GAG CAA GCG TTA GCC GAT CTC AAA AAT TTC TCA AAG GAG CAA TTG Val Glu Gln Ala Leu Ala Asp Leu Lys Asn Phe Ser Lys Glu Gln Leu 805 810 815	3525
GCT CAA CAA GCT CAA AAA AAT GAA AGT TTC AAT GTT GGA AAA TCT GAA Ala Gln Gln Ala Gln Lys Asn Glu Ser Phe Asn Val Gly Lys Ser Glu 820 825 830	3573
ATA TAC CAA TCC GTT AAG AAT GGT GTG AAC GGA ACC CTA GTC GGT AAT Ile Tyr Gln Ser Val Lys Asn Gly Val Asn Gly Thr Leu Val Gly Asn 835 840 845 850	3621
GGG TTA TCT GGA ATA GAG GCC ACA GGG Gly Leu Ser Gly Ile Glu Ala Thr Gly 855	3648

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 859 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Thr Asn Glu Thr Ile Asp Gln Gln Pro Gln Thr Glu Ala Ala Phe 1 5 10 15
Asn Pro Gln Gln Phe Ile Asn Asn Leu Gln Val Ala Phe Leu Lys Val 20 25 30
Asp Asn Ala Val Ala Ser Tyr Asp Pro Asp Gln Lys Pro Ile Val Asp 35 40 45

Lys Asn Asp Arg Asp Asn Arg Gln Ala Phe Glu Gly Ile Ser Gln Leu
 50 55 60

Arg Glu Glu Tyr Ser Asn Lys Ala Ile Lys Asn Pro Thr Lys Lys Asn
 65 70 75 80

Gln Tyr Phe Ser Asp Phe Ile Asn Lys Ser Asn Asp Leu Ile Asn Lys
 85 90 95

Asp Asn Leu Ile Val Val Glu Ser Ser Thr Lys Ser Phe Gln Lys Phe
 100 105 110

Gly Asp Gln Arg Tyr Arg Ile Phe Thr Ser Trp Val Ser His Gln Asn
 115 120 125

Asp Pro Ser Lys Ile Asn Thr Arg Cys Ile Arg Asn Phe Met Glu His
 130 135 140

Thr Ile Gln Pro Pro Ile Pro Asp Asp Lys Glu Lys Ala Glu Phe Leu
 145 150 155 160

Lys Ser Ala Lys Gln Ser Phe Ala Gly Ile Ile Ile Gly Asn Gln Ile
 165 170 175

Arg Thr Asp Gln Lys Phe Met Gly Val Phe Asp Glu Ser Leu Lys Glu
 180 185 190

Arg Gln Glu Ala Glu Lys Asn Gly Gly Pro Thr Gly Gly Asp Trp Leu
 195 200 205

Asp Ile Phe Leu Ser Phe Ile Phe Asp Lys Lys Gln Ser Ser Asp Val
 210 215 220

Lys Glu Ala Ile Asn Gln Glu Pro Leu Pro His Val Gln Pro Asp Ile
 225 230 235 240

Ala Thr Ser Thr Thr His Ile Gln Gly Leu Pro Pro Glu Ser Arg Asp
 245 250 255

Leu Leu Asp Glu Arg Gly Asn Phe Ser Lys Phe Thr Leu Gly Asp Met
 260 265 270

Glu Met Leu Asp Val Glu Gly Val Ala Asp Met Asp Pro Asn Tyr Lys
 275 280 285

Phe Asn Gln Leu Leu Ile His Asn Asn Thr Leu Ser Ser Val Leu Met
 290 295 300

Gly Ser His Asp Gly Ile Glu Pro Glu Lys Val Ser Leu Leu Tyr Ala
 305 310 315 320

Gly Asn Gly Gly Phe Gly Ala Lys His Asp Trp Asn Ala Thr Val Gly
 325 330 335

Tyr Lys Asp Gln Gln Gly Asn Asn Val Ala Thr Ile Ile Asn Val His
 340 345 350

60

Met Lys Asn Gly Ser Gly Leu Val Ile Ala Gly Gly Glu Lys Gly Ile
355 360 365

Asn Asn Pro Ser Phe Tyr Leu Tyr Lys Glu Asp Gln Leu Thr Gly Ser
370 375 380

Gln Arg Ala Leu Ser Gln Glu Glu Ile Gln Asn Lys Ile Asp Phe Met
385 390 395 400

Glu Phe Leu Ala Gln Asn Asn Ala Lys Leu Asp Ser Leu Ser Glu Lys
405 410 415

Glu Lys Glu Lys Phe Arg Asn Glu Ile Lys Asp Phe Gln Lys Asp Ser
420 425 430

Lys Pro Tyr Leu Asp Ala Leu Gly Asn Asp Arg Ile Ala Phe Val Ser
435 440 445

Lys Lys Asp Pro Lys His Ser Ala Leu Ile Thr Glu Phe Asn Lys Gly
450 455 460

Asp Leu Ser Tyr Thr Leu Lys Val Met Gly Lys Lys Gln Ile Lys Ala
465 470 475 480

Leu Asp Arg Glu Lys Asn Val Thr Leu Gln Gly Asn Leu Lys His Asp
485 490 495

Gly Val Met Phe Val Asn Tyr Ser Asn Phe Lys Tyr Thr Asn Ala Ser
500 505 510

Lys Ser Pro Asn Lys Gly Val Gly Val Thr Asn Gly Val Ser His Leu
515 520 525

Glu Ala Gly Phe Ser Lys Val Ala Val Phe Asn Leu Pro Asn Leu Asn
530 535 540

Asn Leu Ala Ile Thr Ser Val Val Arg Arg Asp Leu Glu Asp Lys Leu
545 550 555 560

Ile Ala Lys Gly Leu Ser Pro Gln Glu Ala Asn Lys Leu Val Lys Asp
565 570 575

Phe Leu Ser Ser Asn Lys Glu Leu Val Gly Lys Ala Leu Asn Phe Asn
580 585 590

Lys Ala Val Ala Glu Ala Lys Asn Thr Gly Asn Tyr Asp Glu Val Lys
595 600 605

Arg Ala Gln Lys Asp Leu Glu Lys Ser Leu Lys Lys Arg Glu His Leu
610 615 620

Glu Lys Asp Val Ala Lys Asn Leu Glu Ser Lys Ser Gly Asn Lys Asn
625 630 635 640

Lys Met Glu Ala Lys Ala Gln Ala Asn Ser Gln Lys Asp Glu Ile Phe
645 650 655

Ala Leu Ile Asn Lys Glu Ala Asn Arg Asp Ala Arg Ala Ile Ala Tyr
660 665 670

Ala Gln Asn Leu Lys Gly Ile Lys Arg Glu Leu Ser Asp Lys Leu Glu
675 680 685

Asn Ile Asn Lys Asp Leu Lys Asp Phe Ser Lys Ser Phe Asp Gly Phe
690 695 700

Lys Asn Gly Lys Asn Lys Asp Phe Ser Lys Ala Glu Glu Thr Leu Lys
705 710 715 720

Ala Leu Lys Gly Ser Val Lys Asp Leu Gly Ile Asn Pro Glu Trp Ile
725 730 735

Ser Lys Val Glu Asn Leu Asn Ala Ala Leu Asn Glu Phe Lys Asn Gly
740 745 750

Lys Asn Lys Asp Phe Ser Lys Val Thr Gln Ala Lys Ser Asp Gln Glu
755 760 765

Asn Ser Ile Lys Asp Val Ile Ile Asn Gln Lys Ile Thr Asp Lys Val
770 775 780

Asp Glu Leu Asn Gln Ala Val Ser Val Ala Lys Ile Ala Cys Asp Phe
785 790 795 800

Ser Gly Val Glu Gln Ala Leu Ala Asp Leu Lys Asn Phe Ser Lys Glu
805 810 815

Gln Leu Ala Gln Gln Ala Gln Lys Asn Glu Ser Phe Asn Val Gly Lys
820 825 830

Ser Glu Ile Tyr Gln Ser Val Lys Asn Gly Val Asn Gly Thr Leu Val
835 840 845

Gly Asn Gly Leu Ser Gly Ile Glu Ala Thr Gly
850 855

(2) INFORMATION FOR SEQ ID NO:3:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4821 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1072..4614

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATGGGCTGCG CGTAACGAAA AACAGTCGCT TGACCTCTT TGATGTCATC AGAGATTTTC	60
CAAATATCCG CTATAACCTTT GACTCCTAGA GCGCAACCAC CTACGATCGC TAGAACAGAA	120
ATGATCTGAA CCACCAAAGT TTTAGTCTCA GTAATGCCTG ATGCAGGACT GTCGAAAGCC	180
ATTAAAGGAT TGGCTGCTAT CGCTAGCCCT AAAGTTACTA CAACTTCTT GTAGCTGTCA	240
GTGATTCTTG TAAAAAAATT CATGCGTTTC CTTCAAATT GAAATCAATC GTTGAGTAT	300
ATCAAAAAAA AGTATTTTA TACTATTCA ACAAGCGCTA CTTTATAATT TAAATCAAAA	360
CCGACGCTTT TGTTTGACAA CTGATATAAT TTAGGAACAA TAAACCTACT TGTCCCAACC	420
ATTTTCTTT CTCAAGTCAT CGTAGAATTG TAGATCTTTA GGATCTTGA TGTATTTTT	480
AATCGTCTCA GGTTGAAACC TAAAAACAAG CAGAAACAAA CCCAAGCTGA TCAGAGTGAG	540
AATAAAGCTC CATTAAAGC AACTCCATAA ACCACTAAAG AAACTTTTTG TGAGACTCTC	600
TTTGAAAATC TGT CCTATTG ATTTGTTTC CATTGGTTT CCCATGCGGA TCACAAACGC	660
TTAATTACAA ATACATACTA TAATAAGTAT GGCACACACAA ACCAAACCA TTTTTAGAAC	720
GCTTCATGCA CTCACCTTGC TCCTAACCAT TTCTCCAACC ATCTTAGCG TTGCATTGAA	780
TTTCTCAAA AAGGCTCATT TCTTAGTTTC TTTATTCTT AAAATTTTC CATTCTAGCA	840
AATTTTGTT AATTGTGGGT AAAAATGTGA ATCGTTCTA GCTTTAGAC GCTTGCAACG	900
ATCGGACTTT TTTCATATT AATGAAAAAA TGCCAAATAT TCTAAATATT GTGGTATAGT	960
GATAACGTTCA AAAGACACGA ATTGCATACT CAAAGTGTGT AGTAGTTTT AGCGGTCTTT	1020
GATACCAATA AGATACCGAT AGGTATGAAA CTAGGTATAG AAGGAGAAAC A ATG ACT Met Thr 1	1077
AAC GAA ACT ATT GAC CAA CAA CCA CAA ACC GAA GCG GCT TTT AAC CCG Asn Glu Thr Ile Asp Gln Gln Pro Gln Thr Glu Ala Ala Phe Asn Pro	1125
5 10 15	
CAG CAA TTT ATC AAT AAT CTT CAA GTA GCT TTT CTT AAA GTT GAT AAC Gln Gln Phe Ile Asn Asn Leu Gln Val Ala Phe Leu Lys Val Asp Asn	1173
20 25 30	
GCT GTC GCT TCA TAC GAT CCT GAT CAA AAA CCA ATC GTT GAT AAG AAC Ala Val Ala Ser Tyr Asp Pro Asp Gln Lys Pro Ile Val Asp Lys Asn	1221
35 40 45 50	
GAT AGG GAT AAC AGG CAA GCT TTT GAG GGA ATC TCG CAA TTA AGG GAA Asp Arg Asp Asn Arg Gln Ala Phe Glu Gly Ile Ser Gln Leu Arg Glu	1269
55 60 65	

GAA TAC TCC AAT AAA GCG ATC AAA AAT CCT ACC AAA AAG AAT CAG TAT Glu Tyr Ser Asn Lys Ala Ile Lys Asn Pro Thr Lys Lys Asn Gin Tyr 70 75 80	1317
TTT TCA GAC TTT ATC AAT AAG AGC AAT GAT TTA ATC AAC AAA GAC AAT Phe Ser Asp Phe Ile Asn Lys Ser Asn Asp Leu Ile Asn Lys Asp Asn 85 90 95	1365
CTC ATT GTC GTG GAA TCT TCC ACA AAG AGC TTT CAG AAA TTT GGG GAT Leu Ile Val Val Glu Ser Ser Thr Lys Ser Phe Gln Lys Phe Gly Asp 100 105 110	1413
CAG CGT TAC CGA ATT TTC ACA AGT TGG GTG TCC CAT CAA AAC GAT CCG Gln Arg Tyr Arg Ile Phe Thr Ser Trp Val Ser His Gln Asn Asp Pro 115 120 125 130	1461
TCT AAA ATC AAC ACC CGA TGC ATC CGA AAT TTT ATG GAA CAT ACC ATA Ser Lys Ile Asn Thr Arg Cys Ile Arg Asn Phe Met Glu His Thr Ile 135 140 145	1509
CAA CCC CCT ATC CCT GAT GAC AAA GAA AAA GCA GAG TTT TTG AAA TCT Gin Pro Pro Ile Pro Asp Asp Lys Glu Lys Ala Glu Phe Leu Lys Ser 150 155 160	1557
GCC AAA CAA TCT TTT GCA GGA ATC ATC ATA GGG AAT CAA ATC CGA ACG Ala Lys Gln Ser Phe Ala Gly Ile Ile Ile Gly Asn Gln Ile Arg Thr 165 170 175	1605
GAT CAA AAA TTC ATG GGC GTG TTT GAT GAA TCC TTG AAA GAA AGG CAA Asp Gln Lys Phe Met Gly Val Phe Asp Glu Ser Leu Lys Glu Arg Gln 180 185 190	1653
GAA GCA GAA AAA AAT GGA GGG CCT ACT GGT GGG GAT TGG TTG GAT ATT Glu Ala Glu Lys Asn Gly Gly Pro Thr Gly Gly Asp Trp Leu Asp Ile 195 200 205 210	1701
TTT TTA TCA TTT ATA TTT GAC AAA AAA CAA TCT TCT GAT GTC AAA GAA Phe Leu Ser Phe Ile Phe Asp Lys Lys Gln Ser Ser Asp Val Lys Glu 215 220 225	1749
GCA ATC AAT CAA GAA CCA CTT CCT CAT GTC CAA CCA GAT ATA GCC ACT Ala Ile Asn Gln Glu Pro Leu Pro His Val Gln Pro Asp Ile Ala Thr 230 235 240	1797
AGC ACC ACT CAC ATA CAA GGC TTA CCG CCT GAA TCT AGG GAT TTG CTT Ser Thr Thr His Ile Gln Gly Leu Pro Pro Glu Ser Arg Asp Leu Leu 245 250 255	1845
GAT GAA AGG GGT AAT TTT TCT AAA TTC ACT CTT GGC GAT ATG GAA ATG Asp Glu Arg Gly Asn Phe Ser Lys Phe Thr Leu Gly Asp Met Glu Met 260 265 270	1893
TTA GAT GTT GAG GGC GTC GCC GAC ATG GAT CCC AAT TAC AAG TTC AAT Leu Asp Val Glu Gly Val Ala Asp Met Asp Pro Asn Tyr Lys Phe Asn 275 280 285 290	1941

CAA TTA TTG ATT CAC AAT AAC ACT CTG TCT GTG TTA ATG GGG AGT Gln Leu Leu Ile His Asn Asn Thr Leu Ser Ser Val Leu Met Gly Ser 295 300 305	1989
CAT GAT GGC ATA GAA CCT GAA AAA GTT TCA TTA TTG TAT GCG GGC AAT His Asp Gly Ile Glu Pro Glu Lys Val Ser Leu Leu Tyr Ala Gly Asn 310 315 320	2037
GGT GGT TTT GGA GCC AAG CAC GAT TGG AAC GCC ACC GTT GGT TAT AAA Gly Gly Phe Gly Ala Lys His Asp Trp Asn Ala Thr Val Gly Tyr Lys 325 330 335	2085
GAC CAA CAA GGT AAC AAT GTG GCT ACA ATA ATT AAT GTG CAT ATG AAA Asp Gln Gln Gly Asn Asn Val Ala Thr Ile Ile Asn Val His Met Lys 340 345 350	2133
AAC GGC AGT GGC TTA GTC ATA GCA GGT GGT GAG AAA GGG ATT AAC AAC Asn Gly Ser Gly Leu Val Ile Ala Gly Gly Glu Lys Gly Ile Asn Asn 355 360 365 370	2181
CCT AGT TTT TAT CTC TAC AAA GAA GAC CAA CTC ACA GGC TCA CAA CGA Pro Ser Phe Tyr Leu Tyr Lys Glu Asp Gln Leu Thr Gly Ser Gln Arg 375 380 385	2229
GCA TTG AGT CAA GAA GAG ATC CAA AAC AAA ATA GAT TTC ATG GAA TTT Ala Leu Ser Gln Glu Glu Ile Gln Asn Lys Ile Asp Phe Met Glu Phe 390 395 400	2277
CTT GCA CAA AAC AAT GCT AAA TTA GAC AGC TTG AGC GAG AAA GAG AAA Leu Ala Gln Asn Asn Ala Lys Leu Asp Ser Leu Ser Glu Lys Glu Lys 405 410 415	2325
GAA AAA TTC CGA AAT GAG ATT AAG GAT TTC CAA AAA GAC TCT AAG CCT Glu Lys Phe Arg Asn Glu Ile Lys Asp Phe Gln Lys Asp Ser Lys Pro 420 425 430	2373
TAT TTA GAC GCC CTA GGG AAT GAT CGT ATT GCT TTT GTT TCT AAA AAA Tyr Leu Asp Ala Leu Gly Asn Asp Arg Ile Ala Phe Val Ser Lys Lys 435 440 445 450	2421
GAC CCA AAA CAT TCA GCT TTA ATT ACT GAG TTT AAT AAG GGG GAT TTG Asp Pro Lys His Ser Ala Leu Ile Thr Glu Phe Asn Lys Gly Asp Leu 455 460 465	2469
AGC TAC ACT CTC AAA GTT ATG GGA AAA AAG CAG ATA AAG GCT TTA GAT Ser Tyr Thr Leu Lys Val Met Gly Lys Lys Gln Ile Lys Ala Leu Asp 470 475 480	2517
AGG GAG AAA AAT GTC ACT CTT CAA GGT AAC CTA AAA CAT GAT GGC GTG Arg Glu Lys Asn Val Thr Leu Gln Gly Asn Leu Lys His Asp Gly Val 485 490 495	2565
ATG TTT GTT AAT TAT TCT AAT TTC AAA TAC ACC AAC GCC TCC AAG AGT Met Phe Val Asn Tyr Ser Asn Phe Lys Tyr Thr Asn Ala Ser Lys Ser 500 505 510	2613

65

CCC AAT AAG GGT GTA GGC GTT ACG AAT GGC GTT TCC CAT TTA GAA GCA Pro Asn Lys Gly Val Gly Val Thr Asn Gly Val Ser His Leu Glu Ala 515 520 525 530	2661
GGC TTT AGC AAG GTG GCT GTC TTT AAT TTG CCT AAT TTA AAT AAT CTC Gly Phe Ser Lys Val Ala Val Phe Asn Leu Pro Asn Leu Asn Asn Leu 535 540 545	2709
GCT ATC ACT AGT GTC GTA AGG CGG GAT TTA GAG GAT AAA CTA ATC GCT Ala Ile Thr Ser Val Val Arg Arg Asp Leu Glu Asp Lys Leu Ile Ala 550 555 560	2757
AAA GGA TTG TCC CCA CAA GAA GCT AAT AAG CTT GTC AAA GAT TTT TTG Lys Gly Leu Ser Pro Gln Glu Ala Asn Lys Leu Val Lys Asp Phe Leu 565 570 575	2805
AGT AGC AAC AAA GAA TTG GTT GGA AAA GCT TTA AAC TTC AAT AAA GCT Ser Ser Asn Lys Glu Leu Val Gly Lys Ala Leu Asn Phe Asn Lys Ala 580 585 590	2853
GTA GCT GAA GCT AAA AAC ACA GGC AAC TAT GAC GAG GTG AAA CGA GCT Val Ala Glu Ala Lys Asn Thr Gly Asn Tyr Asp Glu Val Lys Arg Ala 595 600 605 610	2901
CAG AAA GAT CTT GAA AAA TCT CTA AAG AAA CGA GAG CAT TTG GAG AAG Gln Lys Asp Leu Glu Lys Ser Leu Lys Lys Arg Glu His Leu Glu Lys 615 620 625	2949
GAT GTA GCG AAA AAT TTG GAG AGC AAA AGC GGC AAC AAA AAT AAA ATG Asp Val Ala Lys Asn Leu Glu Ser Lys Ser Gly Asn Lys Asn Lys Met 630 635 640	2997
GAA GCA AAA GCT CAA GCT AAC AGC CAA AAA GAT GAG ATT TTT GCG TTG Glu Ala Lys Ala Gln Ala Asn Ser Gln Lys Asp Glu Ile Phe Ala Leu 645 650 655	3045
ATC AAT AAA GAG GCT AAT AGA GAC GCA AGA GCA ATC GCT TAC GCT CAA Ile Asn Lys Glu Ala Asn Arg Asp Ala Arg Ala Ile Ala Tyr Ala Gln 660 665 670	3093
AAT CTT AAA GGC ATC AAA AGG GAA TTG TCT GAT AAA CTT GAA AAT ATC Asn Leu Lys Gly Ile Lys Arg Glu Leu Ser Asp Lys Leu Glu Asn Ile 675 680 685 690	3141
AAC AAG GAT TTG AAA GAC TTT AGT AAA TCT TTT GAT GGA TTC AAA AAT Asn Lys Asp Leu Lys Asp Phe Ser Lys Ser Phe Asp Gly Phe Lys Asn 695 700 705	3189
GGC AAA AAT AAG GAT TTC AGC AAG GCA GAA GAA ACG CTA AAA GCC CTT Gly Lys Asn Lys Asp Phe Ser Lys Ala Glu Glu Thr Leu Lys Ala Leu 710 715 720	3237
AAA GGC TCG GTG AAA GAT TTG GGT ATC AAT CCG GAA TGG ATT TCA AAA Lys Gly Ser Val Lys Asp Leu Gly Ile Asn Pro Glu Trp Ile Ser Lys 725 730 735	3285

GTT GAA AAC CTT AAT GCA GCT TTG AAT GAA TTC AAA AAT GGC AAA AAT Val Glu Asn Leu Asn Ala Ala Leu Asn Glu Phe Lys Asn Gly Lys Asn 740 745 750	3333
AAG GAT TTC AGC AAG GTA ACG CAA GCA AAA AGC GAC CAA GAA AAT TCC Lys Asp Phe Ser Lys Val Thr Gln Ala Lys Ser Asp Gln Glu Asn Ser 755 760 765 770	3381
ATT AAA GAT GTG ATC ATC AAT CAA AAG ATA ACG GAT AAA GTT GAT GAA Ile Lys Asp Val Ile Ile Asn Gln Lys Ile Thr Asp Lys Val Asp Glu 775 780 785	3429
CTC AAT CAA GCG GTA TCA GTG GCT AAA ATA GCG TGC GAT TTC AGT GGG Leu Asn Gln Ala Val Ser Val Ala Lys Ile Ala Cys Asp Phe Ser Gly 790 795 800	3477
GTA GAG CAA GCG TTA GCC GAT CTC AAA AAT TTC TCA AAG GAG CAA TTG Val Glu Gln Ala Leu Ala Asp Leu Lys Asn Phe Ser Lys Glu Gln Leu 805 810 815	3525
GCT CAA CAA GCT CAA AAA AAT GAA AGT TTC AAT GTT GGA AAA TCT GAA Ala Gln Gln Ala Gln Lys Asn Glu Ser Phe Asn Val Gly Lys Ser Glu 820 825 830	3573
ATA TAC CAA TCC GTT AAG AAT GGT GTG AAC GGA ACC CTA GTC GGT AAT Ile Tyr Gln Ser Val Lys Asn Gly Val Asn Gly Thr Leu Val Gly Asn 835 840 845 850	3621
GGG TTA TCT GGA ATA GAG GCC ACA GCT CTC GCC AAA AAT TTT TCG GAT Gly Leu Ser Gly Ile Glu Ala Thr Ala Leu Ala Lys Asn Phe Ser Asp 855 860 865	3669
ATC AAG AAA GAA TTG AAT GAG AAA TTT AAA AAT TTC AAT AAC AAT AAC Ile Lys Lys Glu Leu Asn Glu Lys Phe Lys Asn Phe Asn Asn Asn Asn 870 875 880	3717
AAT AAT GGT CTC AAA AAC GGC GGA GAA CCC ATT TAT GCT CAA GTT AAT Asn Asn Gly Leu Lys Asn Gly Gly Glu Pro Ile Tyr Ala Gln Val Asn 885 890 895	3765
AAA AAG AAA ACA GGA CAA GTA GCT AGC CCT GAA GAA CCC ATT TAT GCT Lys Lys Lys Thr Gly Gln Val Ala Ser Pro Glu Glu Pro Ile Tyr Ala 900 905 910	3813
CAA GTT GCT AAA AAG GTA ACT AAA AAA ATT GAC CAA CTC AAT CAA GCA Gln Val Ala Lys Lys Val Thr Lys Lys Ile Asp Gln Leu Asn Gln Ala 915 920 925 930	3861
GCG ACA AGT GGT TTC GGT GGT GTA GGG CAA GCG GGC TTC CCT TTG AAA Ala Thr Ser Gly Phe Gly Val Gly Gln Ala Gly Phe Pro Leu Lys 935 940 945	3909
AGG CAT GAT AAA GTT GAA GAT CTC AGT AAG GTA GGG CGA TCA GTT AGC Arg His Asp Lys Val Glu Asp Leu Ser Lys Val Gly Arg Ser Val Ser 950 955 960	3957

CCT GAA CCC ATT TAT GCT ACA ATT GAT GAT CTC GGT GGG TCT TTC CCT Pro Glu Pro Ile Tyr Ala Thr Ile Asp Asp Leu Gly Gly Ser Phe Pro 965 970 975	4005
TTG AAA AGG CAT GAT AAA GTT GAT GAT CTC AGT AAG GTA GGG CTT TCA Leu Lys Arg His Asp Lys Val Asp Asp Leu Ser Lys Val Gly Leu Ser 980 985 990	4053
AGG AAT CAA GAA TTG ACT CAG AAA ATT GAC AAT CTC AGT CAA GCG GTA Arg Asn Gln Glu Leu Thr Gln Lys Ile Asp Asn Leu Ser Gln Ala Val 995 1000 1005 1010	4101
TCA GAA GCT AAA GCA GGT TTT TTT GGC AAT CTA GAA CAA ACG ATA GAC Ser Glu Ala Lys Ala Gly Phe Phe Gly Asn Leu Glu Gln Thr Ile Asp 1015 1020 1025	4149
AAG CTC AAA GAT TTT ACA AAA AAC AAT CCT GTG AAT CTA TGG GCT GAA Lys Leu Lys Asp Phe Thr Lys Asn Asn Pro Val Asn Leu Trp Ala Glu 1030 1035 1040	4197
AGC GCA AAA AAA GTG CCT GCT AGT TTG TCA GCG AAA CTA GAC AAT TAC Ser Ala Lys Lys Val Pro Ala Ser Leu Ser Ala Lys Leu Asp Asn Tyr 1045 1050 1055	4245
GCT ACT AAC AGC CAC ACA CGC ATT AAT AGC AAT ATC CAA AAT GGA GCG Ala Thr Asn Ser His Thr Arg Ile Asn Ser Asn Ile Gln Asn Gly Ala 1060 1065 1070	4293
ATC AAT GAA AAA GCG ACC GGC ACT GAA CGG CAA AAA AAC CCT GAG TGG Ile Asn Glu Lys Ala Thr Gly Thr Glu Arg Gln Lys Asn Pro Glu Trp 1075 1080 1085 1090	4341
CTC AAA CTC GTG AAT GAT AAG ATC GTT GCG CAT AAT GTG GGA AGC GTT Leu Lys Leu Val Asn Asp Lys Ile Val Ala His Asn Val Gly Ser Val 1095 1100 1105	4389
CCT TTG TCA GAG TAT GAT AAC ATT GGA TTC AGC CAA AAG AAT ATG AAG Pro Leu Ser Glu Tyr Asp Asn Ile Gly Phe Ser Gln Lys Asn Met Lys 1110 1115 1120	4437
GAT TAT TCT GAT TCG TTC AAG TTT TCC ACC AAG TTG AAC AAT GCC GTA Asp Tyr Ser Asp Ser Phe Lys Phe Ser Thr Lys Leu Asn Asn Ala Val 1125 1130 1135	4485
AAA GAC ATT AAG TCT GGC TTT ACG CAA TTT TTA GCC AAT GCA TTT TCT Lys Asp Ile Lys Ser Gly Phe Thr Gln Phe Leu Ala Asn Ala Phe Ser 1140 1145 1150	4533
ACA GGA TAT TAC TCC ATG GCG AGA GAA AAT GCG GAG CAT GGA ATC AAA Thr Gly Tyr Tyr Ser Met Ala Arg Glu Asn Ala Glu His Gly Ile Lys 1155 1160 1165 1170	4581
AAT GCT AAT ACA AAA GGT GGT TTC CAA AAA TCT TAAAGGATTA AGGAACACCA Asn Ala Asn Thr Lys Gly Gly Phe Gln Lys Ser 1175 1180	4634

AAAACGCAA AACCACCTTG CTAAAAGCAA GGGGTTTTT AACTAAAAT ATCCCGACAG	4694
ACACTAACGA AAGGCTTTGT TCTTTAAAGT CTGCATAGAT ATTCCTTACCC CCAAAAAGAC	4754
TTAACCCCTT GCTTAAAATT AAATTGATT GTGCTAGTGG GTTCGTGCTT TATAGTGCAG	4814
AATTGGG	4821

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1181 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Thr Asn Glu Thr Ile Asp Gln Gln Pro Gln Thr Glu Ala Ala Phe			
1	5	10	15
Asn Pro Gln Gln Phe Ile Asn Asn Leu Gln Val Ala Phe Leu Lys Val			
20	25	30	
Asp Asn Ala Val Ala Ser Tyr Asp Pro Asp Gln Lys Pro Ile Val Asp			
35	40	45	
Lys Asn Asp Arg Asp Asn Arg Gln Ala Phe Glu Gly Ile Ser Gln Leu			
50	55	60	
Arg Glu Glu Tyr Ser Asn Lys Ala Ile Lys Asn Pro Thr Lys Lys Asn			
65	70	75	80
Gln Tyr Phe Ser Asp Phe Ile Asn Lys Ser Asn Asp Leu Ile Asn Lys			
85	90	95	
Asp Asn Leu Ile Val Val Glu Ser Ser Thr Lys Ser Phe Gln Lys Phe			
100	105	110	
Gly Asp Gln Arg Tyr Arg Ile Phe Thr Ser Trp Val Ser His Gln Asn			
115	120	125	
Asp Pro Ser Lys Ile Asn Thr Arg Cys Ile Arg Asn Phe Met Glu His			
130	135	140	
Thr Ile Gln Pro Pro Ile Pro Asp Asp Lys Glu Lys Ala Glu Phe Leu			
145	150	155	160
Lys Ser Ala Lys Gln Ser Phe Ala Gly Ile Ile Ile Gly Asn Gln Ile			
165	170	175	
Arg Thr Asp Gln Lys Phe Met Gly Val Phe Asp Glu Ser Leu Lys Glu			
180	185	190	

Arg Gln Glu Ala Glu Lys Asn Gly Gly Pro Thr Gly Gly Asp Trp Leu
195 200 205

Asp Ile Phe Leu Ser Phe Ile Phe Asp Lys Lys Gln Ser Ser Asp Val
210 215 220

Lys Glu Ala Ile Asn Gln Glu Pro Leu Pro His Val Gln Pro Asp Ile
225 230 235 240

Ala Thr Ser Thr Thr His Ile Gln Gly Leu Pro Pro Glu Ser Arg Asp
245 250 255

Leu Leu Asp Glu Arg Gly Asn Phe Ser Lys Phe Thr Leu Gly Asp Met
260 265 270

Glu Met Leu Asp Val Glu Gly Val Ala Asp Met Asp Pro Asn Tyr Lys
275 280 285

Phe Asn Gln Leu Leu Ile His Asn Asn Thr Leu Ser Ser Val Leu Met
290 295 300

Gly Ser His Asp Gly Ile Glu Pro Glu Lys Val Ser Leu Leu Tyr Ala
305 310 315 320

Gly Asn Gly Gly Phe Gly Ala Lys His Asp Trp Asn Ala Thr Val Gly
325 330 335

Tyr Lys Asp Gln Gln Gly Asn Asn Val Ala Thr Ile Ile Asn Val His
340 345 350

Met Lys Asn Gly Ser Gly Leu Val Ile Ala Gly Gly Glu Lys Gly Ile
355 360 365

Asn Asn Pro Ser Phe Tyr Leu Tyr Lys Glu Asp Gln Leu Thr Gly Ser
370 375 380

Gln Arg Ala Leu Ser Gln Glu Glu Ile Gln Asn Lys Ile Asp Phe Met
385 390 395 400

Glu Phe Leu Ala Gln Asn Asn Ala Lys Leu Asp Ser Leu Ser Glu Lys
405 410 415

Glu Lys Glu Lys Phe Arg Asn Glu Ile Lys Asp Phe Gln Lys Asp Ser
420 425 430

Lys Pro Tyr Leu Asp Ala Leu Gly Asn Asp Arg Ile Ala Phe Val Ser
435 440 445

Lys Lys Asp Pro Lys His Ser Ala Leu Ile Thr Glu Phe Asn Lys Gly
450 455 460

Asp Leu Ser Tyr Thr Leu Lys Val Met Gly Lys Lys Gln Ile Lys Ala
465 470 475 480

Leu Asp Arg Glu Lys Asn Val Thr Leu Gln Gly Asn Leu Lys His Asp
485 490 495

Gly Val Met Phe Val Asn Tyr Ser Asn Phe Lys Tyr Thr Asn Ala Ser
500 505 510

Lys Ser Pro Asn Lys Gly Val Gly Val Thr Asn Gly Val Ser His Leu
515 520 525

Glu Ala Gly Phe Ser Lys Val Ala Val Phe Asn Leu Pro Asn Leu Asn
530 535 540

Asn Leu Ala Ile Thr Ser Val Val Arg Arg Asp Leu Glu Asp Lys Leu
545 550 555 560

Ile Ala Lys Gly Leu Ser Pro Gln Glu Ala Asn Lys Leu Val Lys Asp
565 570 575

Phe Leu Ser Ser Asn Lys Glu Leu Val Gly Lys Ala Leu Asn Phe Asn
580 585 590

Lys Ala Val Ala Glu Ala Lys Asn Thr Gly Asn Tyr Asp Glu Val Lys
595 600 605

Arg Ala Gln Lys Asp Leu Glu Lys Ser Leu Lys Lys Arg Glu His Leu
610 615 620

Glu Lys Asp Val Ala Lys Asn Leu Glu Ser Lys Ser Gly Asn Lys Asn
625 630 635 640

Lys Met Glu Ala Lys Ala Gln Ala Asn Ser Gln Lys Asp Glu Ile Phe
645 650 655

Ala Leu Ile Asn Lys Glu Ala Asn Arg Asp Ala Arg Ala Ile Ala Tyr
660 665 670

Ala Gln Asn Leu Lys Gly Ile Lys Arg Glu Leu Ser Asp Lys Leu Glu
675 680 685

Asn Ile Asn Lys Asp Leu Lys Asp Phe Ser Lys Ser Phe Asp Gly Phe
690 695 700

Lys Asn Gly Lys Asn Lys Asp Phe Ser Lys Ala Glu Glu Thr Leu Lys
705 710 715 720

Ala Leu Lys Gly Ser Val Lys Asp Leu Gly Ile Asn Pro Glu Trp Ile
725 730 735

Ser Lys Val Glu Asn Leu Asn Ala Ala Leu Asn Glu Phe Lys Asn Gly
740 745 750

Lys Asn Lys Asp Phe Ser Lys Val Thr Gln Ala Lys Ser Asp Gln Glu
755 760 765

Asn Ser Ile Lys Asp Val Ile Ile Asn Gln Lys Ile Thr Asp Lys Val
770 775 780

Asp Glu Leu Asn Gln Ala Val Ser Val Ala Lys Ile Ala Cys Asp Phe
785 790 795 800

Ser Gly Val Glu Gln Ala Leu Ala Asp Leu Lys Asn Phe Ser Lys Glu
 805 810 815
 Gln Leu Ala Gln Gln Ala Gln Lys Asn Glu Ser Phe Asn Val Gly Lys
 820 825 830
 Ser Glu Ile Tyr Gln Ser Val Lys Asn Gly Val Asn Gly Thr Leu Val
 835 840 845
 Gly Asn Gly Leu Ser Gly Ile Glu Ala Thr Ala Leu Ala Lys Asn Phe
 850 855 860
 Ser Asp Ile Lys Lys Glu Leu Asn Glu Lys Phe Lys Asn Phe Asn Asn
 865 870 875 880
 Asn Asn Asn Asn Gly Leu Lys Asn Gly Gly Glu Pro Ile Tyr Ala Gln
 885 890 895
 Val Asn Lys Lys Lys Thr Gly Gln Val Ala Ser Pro Glu Glu Pro Ile
 900 905 910
 Tyr Ala Gln Val Ala Lys Lys Val Thr Lys Lys Ile Asp Gln Leu Asn
 915 920 925
 Gln Ala Ala Thr Ser Gly Phe Gly Gly Val Gly Gln Ala Gly Phe Pro
 930 935 940
 Leu Lys Arg His Asp Lys Val Glu Asp Leu Ser Lys Val Gly Arg Ser
 945 950 955 960
 Val Ser Pro Glu Pro Ile Tyr Ala Thr Ile Asp Asp Leu Gly Gly Ser
 965 970 975
 Phe Pro Leu Lys Arg His Asp Lys Val Asp Asp Leu Ser Lys Val Gly
 980 985 990
 Leu Ser Arg Asn Gln Glu Leu Thr Gln Lys Ile Asp Asn Leu Ser Gln
 995 1000 1005
 Ala Val Ser Glu Ala Lys Ala Gly Phe Phe Gly Asn Leu Glu Gln Thr
 1010 1015 1020
 Ile Asp Lys Leu Lys Asp Phe Thr Lys Asn Asn Pro Val Asn Leu Trp
 1025 1030 1035 1040
 Ala Glu Ser Ala Lys Lys Val Pro Ala Ser Leu Ser Ala Lys Leu Asp
 1045 1050 1055
 Asn Tyr Ala Thr Asn Ser His Thr Arg Ile Asn Ser Asn Ile Gln Asn
 1060 1065 1070
 Gly Ala Ile Asn Glu Lys Ala Thr Gly Thr Glu Arg Gln Lys Asn Pro
 1075 1080 1085
 Glu Trp Leu Lys Leu Val Asn Asp Lys Ile Val Ala His Asn Val Gly
 1090 1095 1100

72

Ser Val Pro Leu Ser Glu Tyr Asp Asn Ile Gly Phe Ser Gln Lys Asn
1105 1110 1115 1120

Met Lys Asp Tyr Ser Asp Ser Phe Lys Phe Ser Thr Lys Leu Asn Asn
1125 1130 1135

Ala Val Lys Asp Ile Lys Ser Gly Phe Thr Gln Phe Leu Ala Asn Ala
1140 1145 1150

Phe Ser Thr Gly Tyr Tyr Ser Met Ala Arg Glu Asn Ala Glu His Gly
1155 1160 1165

Ile Lys Asn Ala Asn Thr Lys Gly Gly Phe Gln Lys Ser
1170 1175 1180

What is claimed is:

1. An isolated nucleic acid encoding an approximately 120-128 kilodalton antigen of Helicobacter pylori, or an antigenic fragment thereof, wherein the antigen is associated with peptic ulceration.
2. The nucleic acid of claim 1, comprising nucleotides 1072 through 3648 contained in the nucleotide sequence defined in the Sequencing Listing as SEQ ID NO: 1.
3. A purified antigenic polypeptide fragment encoded by a portion of the nucleic acid of claim 1.
4. The antigenic polypeptide of claim 3, wherein the polypeptide consists essentially of the amino acids encoded by nucleotides 1072 through 3648 contained in the nucleotide sequence defined in the Sequence Listing as SEQ ID NO: 1.
5. A vector comprising the nucleic acid of claim 1.
6. The vector of claim 5 in a host capable of expressing the antigen.
7. A purified monoclonal antibody specifically reactive with the polypeptide encoded by the nucleic acid of Claim 1.
8. A method of detecting the presence of a Helicobacter pylori strain possessing the 120-128 kilodalton antigen in a subject, comprising the steps of:
 - a. contacting an antibody-containing sample from the subject with a detectable amount of the fragment of claim 3;

b. detecting the reaction of the fragment and the antibody, the reaction indicating the presence of the Helicobacter pylori strain.

9. A method of detecting predisposition to peptic ulceration in a subject, comprising the steps of:

a. contacting an antibody-containing sample from the subject with a detectable amount of the fragment of claim 3;

b. detecting the reaction of the fragment and the antibody, the reaction indicating a predisposition of the subject to peptic ulceration.

10. A method of detecting the presence of a Helicobacter pylori strain possessing the 120-128 kilodalton antigen in a subject, comprising the steps of:

a. contacting a sample suspected of containing the 120-128 kilodalton antigen from the subject with a detectable amount of the monoclonal antibody of claim 7; and

b. detecting the reaction of the 120-128 kilodalton antigen and the antibody, the reaction indicating the presence of Helicobacter pylori.

11. A method of detecting predisposition to peptic ulceration in a subject comprising the steps of:

a. contacting a sample suspected of containing the 120-128 kilodalton antigen from the subject with a detectable amount of the monoclonal antibody of claim 7; and

b. detecting the reaction of the 120-128 kilodalton antigen and the antibody, the reaction indicating a predisposition of the subject to peptic ulceration.

12. A method of detecting the presence of the Helicobacter pylori associated with peptic ulceration in a subject, comprising detecting the presence of the nucleic acid of claim 1.

13. The method of claim 12, wherein the nucleic acid is detected utilizing a nucleic acid amplification technique.

14. The method of claim 13 wherein the amplification technique is polymerase chain reaction.

15. The method of claim 12, wherein the nucleic acid is detected utilizing direct hybridization.

16. The method of claim 12, wherein the nucleic acid is detected utilizing a restriction fragment length polymorphism.

17. A method of treating peptic ulcers in a subject, comprising administering to the subject an amount of ligand specifically reactive with the approximately 120-128 kilodalton antigen of Helicobacter pylori sufficient to bind the antigen in the subject and improve the subject's clinical condition.

18. The method of claim 17 wherein the ligand is an antibody.

19. A method of treating peptic ulcers in a subject, comprising administering to the subject an amount of a ligand of a receptor for the 120-128 kilodalton antigen of Helicobacter pylori to react with the receptor and prevent

the binding of the antigen to the receptor to result in improvement in the subject's clinical condition.

20. An immunogenic amount of the fragment of claim 3 in a pharmaceutically acceptable carrier.

21. An isolated nucleic acid capable of selectively hybridizing with or selectively amplifying the nucleic acid of claim 1.

22. An isolated nucleic acid complementary to the nucleic acid of claim 21.

23. A mutant Helicobacter pylori in which the product of the nucleic acid of Claim 1 has been rendered nonfunctional.

24. The Helicobacter pylori strain of claim 23, wherein the Helicobacter pylori is deposited with the American Type Culture Collection under ATCC Accession Number 55359.

25. An immunogenic amount of the mutant Helicobacter pylori of claim 23 in a pharmaceutically acceptable carrier.

26. An antagonist of a receptor for the 120-128 kilodalton antigen of Helicobacter pylori.

27. The nucleic acid of claim 1, comprising nucleotides 1072 through 4614 contained in the nucleotide sequence defined in the Sequencing Listing as SEQ ID NO:3.

28. A purified antigenic polypeptide encoded by a portion of the nucleic acid of claim 27.

29. The antigenic polypeptide of claim 28, wherein the polypeptide consists essentially of the amino acids

encoded by nucleotides 1072 through 4614 contained in the nucleotide sequence defined in the Sequence Listing as SEQ ID NO:3.

30. A vector comprising the nucleic acid of claim 27.

31. The vector of claim 30 in a host capable of expressing the antigen.

32. A purified monoclonal antibody specifically reactive with the polypeptide encoded by the nucleic acid of Claim 27.

33. A method of detecting the presence of a Helicobacter pylori strain possessing the 120-128 kilodalton antigen in a subject, comprising the steps of:

a. contacting an antibody-containing sample from the subject with a detectable amount of the polypeptide of claim 28;

b. detecting the reaction of the fragment and the antibody, the reaction indicating the presence of the Helicobacter pylori strain.

34. A method of detecting predisposition to peptic ulceration in a subject, comprising the steps of:

a. contacting an antibody-containing sample from the subject with a detectable amount of the fragment of claim 28;

b. detecting the reaction of the fragment and the antibody, the reaction indicating a predisposition of the subject to peptic ulceration.

35. A method of detecting the presence of a Helicobacter pylori strain possessing the 120-128 kilodalton antigen in a subject, comprising the steps of:

a. contacting a sample suspected of containing the 120-128 kilodalton antigen from the subject with a detectable amount of the monoclonal antibody of claim 32; and

b. detecting the reaction of the 120-128 kilodalton antigen and the antibody, the reaction indicating the presence of Helicobacter pylori.

36. A method of detecting predisposition to peptic ulceration in a subject comprising the steps of:

a. contacting a sample suspected of containing the 120-128 kilodalton antigen from the subject with a detectable amount of the monoclonal antibody of claim 32; and

b. detecting the reaction of the 120-128 kilodalton antigen and the antibody, the reaction indicating a predisposition of the subject to peptic ulceration.

37. A method of detecting the presence of the Helicobacter pylori associated with peptic ulceration in a subject, comprising detecting the presence of the nucleic acid of claim 27.

38. The method of claim 37, wherein the nucleic acid is detected utilizing a nucleic acid amplification technique.

39. The method of claim 38, wherein the amplification technique is polymerase chain reaction.

40. The method of claim 37, wherein the nucleic acid is detected utilizing direct hybridization.
41. The method of claim 37, wherein the nucleic acid is detected utilizing a restriction fragment length polymorphism.
42. An immunogenic amount of the polypeptide of claim 28 in a pharmaceutically acceptable carrier.
43. An isolated nucleic acid capable of selectively hybridizing with or selectively amplifying the nucleic acid of claim 27.
44. An isolated nucleic acid complementary to the nucleic acid of claim 43.
45. An antagonist of a receptor for the polypeptide of claim 28.

FIGURE 1

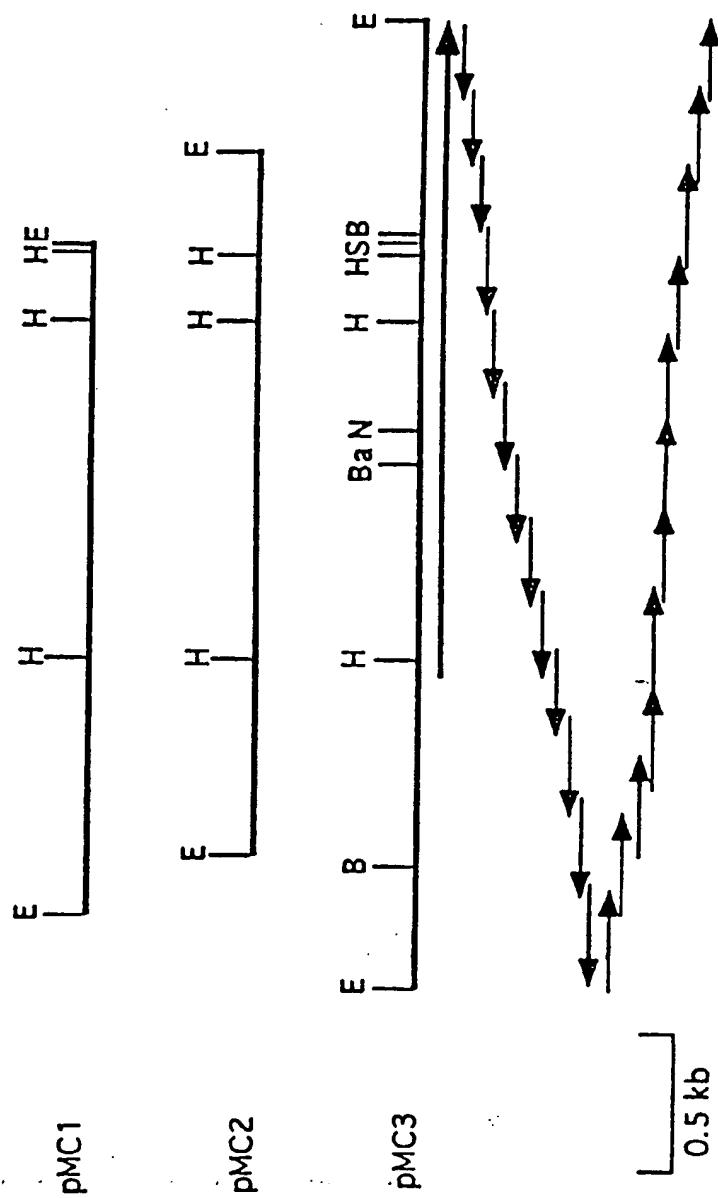
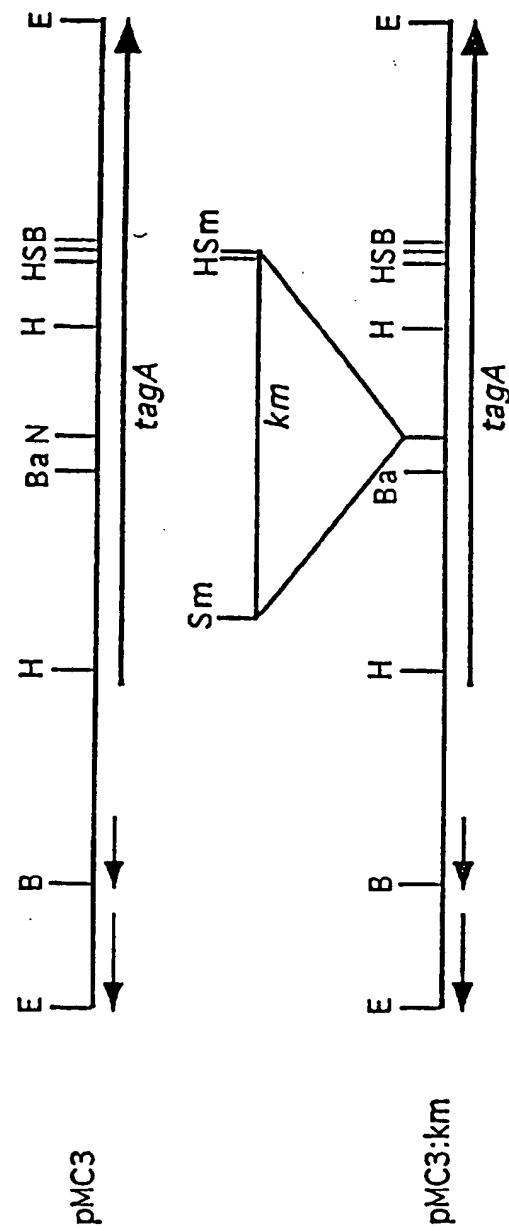
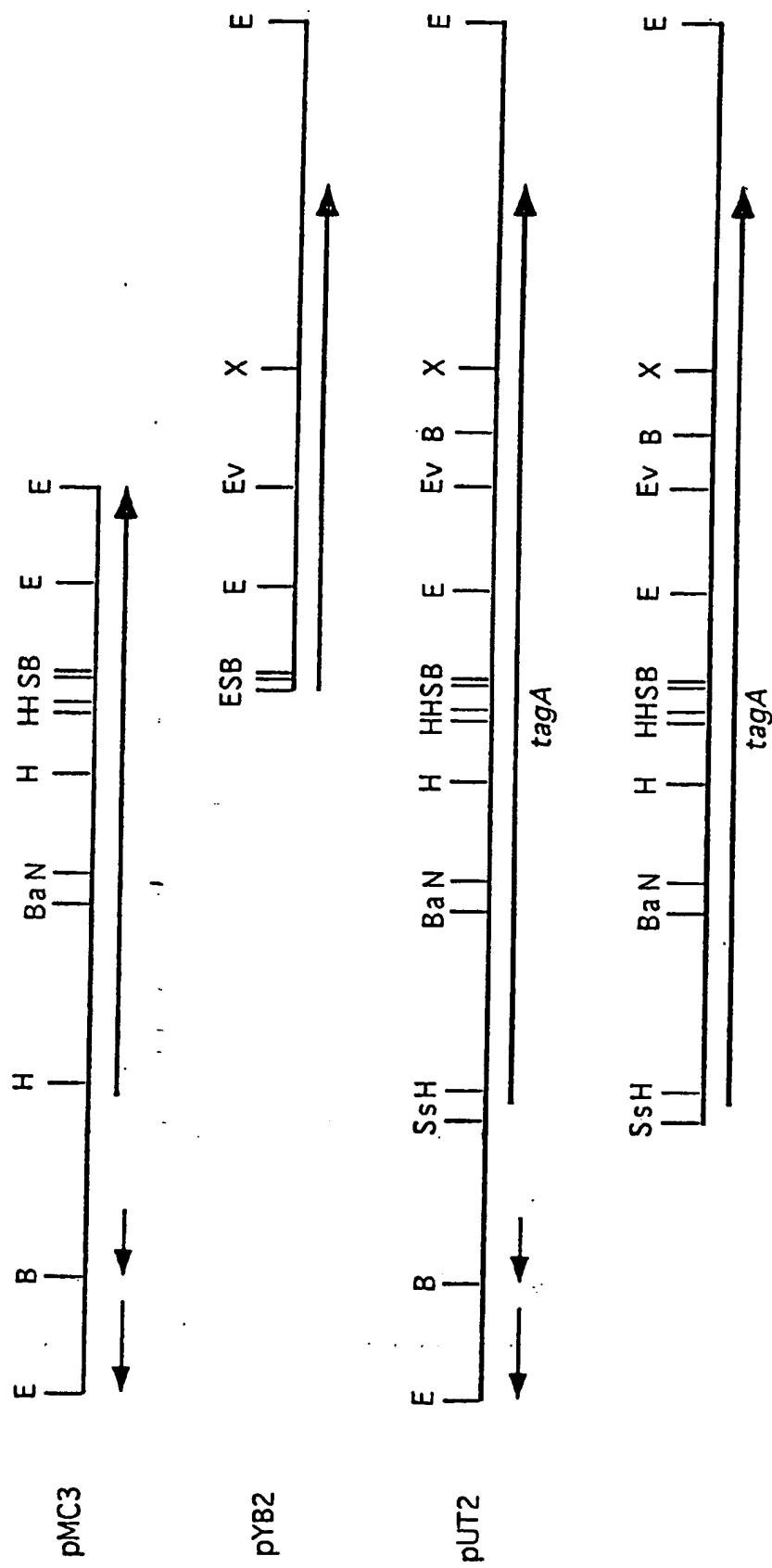


FIGURE 2



3/3

FIGURE 3



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US93/09782

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :Please See Extra Sheet.

US CL :536/23.1, 23.2, 23.7, 24.3, 435/6, 7.1, 91.2; 530/388.2, 388.22

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1, 23.2, 23.7, 24.3, 435/6, 7.1, 91.2; 530/388.2, 388.22

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE, MEDLINE, BIOSIS, APS.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GASTROENTEROLOGY, VOLUME 102, ISSUED 1992, PREWETT ET AL, "DNA PATTERNS OF <u>HELICOBACTER PYLORI</u> ISOLATED FROM GASTRIC ANTRUM, BODY, AND DUODENUM", PAGES 829-833, SEE MATERIALS AND METHODS, PAGE 829 AND FIGURES 1-2.	1,2,21,22,27,43, 44
X	JOURNAL OF BACTERIOLOGY, ISSUED MARCH 1991, LABIGNE ET AL, "SHUTTLE CLONING AND NUCLEOTIDE SEQUENCES OF <u>HELICOBACTER PYLORI</u> GENES RESPONSIBLE FOR UREASE ACTIVITY", PAGES 1920-1931, SEE PAGES 1920-1922.	1,2,5,6,21,22,27, 30,31,43,44
Y	ZBL. BAKT. HYG, ISSUED 1988, APEL ET AL, "ANTIBODY RESPONSE OF PATIENTS AGAINST A 120KDA SURFACE PROTEIN OF <u>CAMPYLOBACTER PYLORI</u> ", PAGES 271-276, SEE PAGES 274-275.	7,8,9,10,11,17,1 8,19,20,26,29,32 ,34,35,36,45

 Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:	T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"		document defining the general state of the art which is not considered to be part of particular relevance
"B"		earlier document published on or after the international filing date
"L"		document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O"		document referring to an oral disclosure, use, exhibition or other means
"P"		document published prior to the international filing date but later than the priority date claimed
"T"		document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"X"		document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"Y"		document member of the same patent family

Date of the actual completion of the international search

29 November 1993

Date of mailing of the international search report

27 DEC.

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. NOT APPLICABLE

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US93/09782

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF CLINICAL MICROBIOLOGY, VOLUME 29, NUMBER 11, HO ET AL, "DIRECT POLYMERASE CHAIN REACTION TEST FOR DETECTION OF <u>HELICOBACTER PYLORI</u> IN HUMANS AND ANIMALS, PAGES 2543-2549, SEE PAGES 2545-2548.	<u>12,13,14,15,38,39,40</u> ,16,21,22,23,27,34,37,41
Y	JOURNAL OF BIOLOGICAL CHEMISTRY, ISSUED 25, MAY 1992, COVER ET AL, "PURIFICATION AND CHARACTERIZATION OF THE VACUOLATING TOXIN FROM <u>HELICOBACTER PYLORI</u> ", PAGES 10570-10575, SEE PAGES 10570-10572 AND 10574.	1-12,15-16,27,37,40,41
X	DIAGN MICROBIOL INFECT DIS, VOLUME 13, HOSHINA ET AL, "DIRECT DETECTION AND AMPLIFICATION OF HELICOBACTER PYLORI RIBOSOMAL 16S GENE SEGMENTS FROM GASTRIC ENDOSCOPIC BIOPSIES", PAGES 473-479, SEE PAGES 475-479.	<u>12-16,38-40,43,1-</u> 6,21,22,24,27,29,30,41,44
Y	JOURNAL OF CLINICAL INVESTIGATION, VOLUME 90, ISSUED SEPTEMBER 1992, COVER ET AL, "SERUM NEUTRALIZING ANTIBODY RESPONSE TO THE VACUOLATING CYTOTOXIN OF <u>HELIBACTER PYLORI</u> ", PAGES 1-6, SEE PAGES 1-2.	3-11,17-20,25-26,28-36,42,45.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US93/09782

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (5):

C07H 21/02, 21/04; A61K 37/66, 39/00, 48/00; C12Q 1/68; C12N 1/20; C07K 15/28; G01N 33/53